(2)

REPORT NO. PNL-6408

TERATOLOGY STUDIES OF LEWISITE AND SULFUR MUSTARD AGENTS: EFFECTS OF LEWISITE IN RATS AND RABBITS

FINAL REPORT

P. L. Hackett, L. B. Sasser, R. L. Rommereim, J. A. Cushing,
R. L. Buschbom and D. R. Kalkwarf

DECEMBER 31, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012

Army Project Order No. 83PP3810

Pacific Northwest Laboratory
P. O. Box 999
Richland, WA 99352-0999

DOD DISTRIBUTION

Approved for public release;
distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

DISCLAIMER

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE MEMORIAL INSTITUTE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

SECURITY CLASSIFICATION OF THIS PAGE

ADA198423

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp. Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE ---	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) DE-AC06076RL0-1830	5. MONITORING ORGANIZATION REPORT NUMBER(S) ---
6a. NAME OF PERFORMING ORGANIZATION Pacific Northwest Laboratory 6b. OFFICE SYMBOL (If applicable) ---	7a. NAME OF MONITORING ORGANIZATION ---
6c. ADDRESS (City, State, and ZIP Code) PO Box 999 Richland, WA 99352-0999	7b. ADDRESS (City, State, and ZIP Code) ---
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical R&D Command 8b. OFFICE SYMBOL (If applicable) ---	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Army Project Order #83PP3810
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63764A; PROJECT NO. 3M46376 40995; TASK NO. AA; WORK UNIT ACCESSION NO 033

11. TITLE (Include Security Classification) Teratology Studies on Lewisite and Sulfur Mustard Agents: Effects of Lewisite in Rats and Rabbits

12. PERSONAL AUTHOR(S) P.L. Hackett, L.B. Sasser, R.L. Rommereim, J.A. Cushing, R.L. Buschbom and D.R. Kalkwarf

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 5/15/83 TO 2/28/87	1987, December 31	65

16. SUPPLEMENTARY NOTATION Project Officer: Robert A. Finch, PhD. US Army Biomedical Research Development Laboratory Fort Detrick, Frederick, MD. Appendices available in Final Report, Part 2 December 31, 1987. Sulfur mustard results are contained in Final Report September 31, 1987.

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Lewisite, Teratology, Rats and Rabbits
06	20		
06	10		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Lewisite was administered to rats and rabbits by intragastric intubation. Rats were dosed daily from 6 through 15 days of gestation (dg) with 0, 0.5, 1.0, 2.0 and 2.5 mg/kg in the dose-range study and with 0, 0.5, 1.0 and 1.5 mg/kg in the teratology study; rabbits were dosed from 6 through 19 dg with 0, 0.5, 1.0, 1.5 and 2.0 mg/kg and 0, 0.07, 0.2 and 0.6 mg/kg in the dose-range and teratology studies, respectively. Maternal animals were weighed periodically, and, at necropsy (20 dg in rats and 30 dg in rabbits), were examined for gross lesions of major organs and reproductive performance; live fetuses were weighed and examined for external, internal and skeletal defects. In rats, a dose level of 1.5 mg/kg did not induce toxic or teratogenic responses in maternal animals or their fetuses. At 2.0 mg/kg, 10% maternal mortality, trends in decreased maternal and fetal body weights and a significant reduction in the number of viable fetuses were evident. In rabbit studies, maternal mortality occurred in all but one of the lewisite treatment groups and

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION
22a. NAME OF RESPONSIBLE INDIVIDUAL Virginia Miller	22b TELEPHONE (Include Area Code) (301) 663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

ranged from 13% to 100% at dose levels of 0.07 and 1.5 mg/kg, respectively. This mortality rate limited the sample size and impaired the detection of statistical significance among treatments. However, at the lowest dose level of the teratology study (0.07 mg/kg), maternal mortality was the only indicator of lewisite toxicity; at the highest dose (0.6 mg/kg), significant findings included 86% maternal mortality, a decrease in maternal body weight gains and an increase in the incidence of fetal stunting, although only a tendency in decreased fetal body weights was observed. These results suggest that maternal mortality was the most important factor in predicting the induction of maternal and fetal effects and, therefore, a "no observable effect level" in maternal animals and their fetuses would be between 1.5 and 2.0 mg/kg in rats and less than 0.07 mg/kg in rabbits.

ACKNOWLEDGEMENT

We wish to express our special appreciation to the following people for their contributions to this project: C. W. Lindenmeir, D. W. Shea and T. A. Breier for administration of the agent and animal manipulations including mating and necropsy; J. E. Evanoff, R. C. Zangar, M. L. Sours, B. J. Willemsen, and B. L. Champion and the members of the Developmental Toxicology Section, who aided in performing the necropsies and fetal evaluations; E. L. Wierman and the staff of the Animal Resources Center for their assistance in maintaining the animals and facility.

DOSE RANGE AND TERATOLOGY STUDIES OF LEWISITE IN RATS AND RABBITS

Quality Assurance Statement

Listed below are the phases and/or procedures included in the study described in this report which were reviewed by the Quality Assurance Unit during the period, 3/1/85 - 10/1/86, specifically for this study and the dates the reviews were performed and findings reported to management. (All findings were reported to the study director or his designee at the time of the review.)

Phase/Procedure Reviewed	Review Date	Date Findings Submitted in Writing to Study Director/Management
Mating	3/14/85	3/20/85
Body Weights	3/19/85	4/12/85
Dosing	3/19/85	4/12/85
Dose Preparation	3/22/85	4/12/85
Data	11/27/85	12/4/85
Animal Identification	2/13/86	3/3/86
Body Weights	2/13/86	3/3/86
Health Screen	2/19/86	3/10/86
Body Weights	2/20/86	3/10/86
Mating	2/20/86	3/10/86
Dosing	3/6/86	3/10/86
Necropsy	3/11/86	3/20/86
Dosing	6/20/86	6/20/86
Body Weights	6/20/86	6/20/86
Artificial Insemination	6/20/86	7/9/86
Clinical Observations	6/20/86	6/20/86
Necropsy	6/10/86	7/10/86
Vehicle Analysis	7/11-12/86	7/22/86
Animal Receipt	7/25/86	7/31/86
Body Weights	8/19/86	8/26/86
Artificial Insemination	8/19/86	8/26/86
Necropsy	9/18/86	9/19/86
Data	3/23-26/87	12/17/87
Final Report	3/23-26/87&12/10,11&17/87	12/17/87

R. C. Lehman
Quality Assurance Specialist

12/17/87
Date

EXECUTIVE SUMMARY

Lewisite, which is a prototype of one of two categories of vesicant war gases, functions by reacting with the sulfhydryl groups of proteins through its arsenic group. Since no information concerning the potential teratogenicity or developmental toxicity of lewisite was available, Pacific Northwest Laboratory, (PNL), under contract with USABRDL, conducted studies to evaluate maternal toxicity, intrauterine mortality and developmental toxicity in rats and rabbits following the administration of this agent by intragastric intubation.

Solutions of lewisite for administration were prepared by diluting the neat agent with sesame oil. Rats were dosed from 6 through 15 days of gestation (dg) with 0, 0.5, 1.0, 2.0 and 2.5 mg/kg in the range-finding study and with 0, 0.5 1.0 and 1.5 mg/kg in the teratology study. Rabbits received from 6 to 19 dg 0, 0.5, 1.0, 1.5 and 2.0 mg/kg and 0, 0.07, 0.2 and 0.6 mg/kg in the dose-range and teratology study, respectively.

Body weights were measured on 0, 6 through 16, and 20 dg in rats, and on 0, 6 through 20, and 30 dg in rabbits. At necropsy (20 dg in rats and 30 dg in rabbits), maternal animals were examined for gross lesions of major organ systems and blood samples were obtained for the measurement of hematocrit levels. Reproductive measures, including numbers of corpora lutea, implantation sites, resorptions and live and dead fetuses were determined. In dose-range studies, live fetuses were weighed and examined for external abnormalities. In the teratology studies, additional examinations of fetal viscera and skeletons were performed to detect morphologic anomalies.

Maternal mortality was 10% and 18% in rats of the two highest dose groups (2.0 and 2.5 mg/kg, respectively) of the dose-range study. The number of live fetuses/litter was significantly decreased at both of these dose levels. Values for maternal and fetal body weights tended to be depressed at a dose level of 2.0 mg/kg and were significantly lower than control values at 2.5 mg/kg. In the teratology study, no evidence of maternal or fetal toxicity, or teratogenicity, was obtained, even at the highest dose level of 1.5 mg/kg.

In rabbit studies, maternal mortality occurred in all but one of the lewisite treatment groups and ranged from 14% at a dose level of 0.07 mg/kg to 100% in animals dosed with 1.5 mg/kg. Probit analysis of mortality data indicated that a dose level to induce 1% lethality would be 0.02 mg/kg (with lower and upper 95% fiducial limits of 0.002 and 0.04, respectively). Determinations of statistically significant differences among treatment groups of the teratology study were limited by the small sample size, which resulted from the unexpectedly high mortality in the lewisite-treated rabbits. In this study, a significant trend in decreasing hematocrit values with increasing lewisite doses was observed. At the highest dose level (0.6 mg/kg), significant findings included a decrease in maternal body weight gains during dosing and an increase in the incidence of fetal stunting, although only a tendency toward decreased fetal body weights was observed.

Large differences between rats and rabbits in dose-response relationships were observed, as exemplified by the LD_{50} values which were calculated to be 3.1 and 0.26 mg/kg, respectively. The LD_{50} value for rats, which was 11.9 times higher than the value for rabbits, may have resulted from the administration of a higher concentration (rat concentration x 13.3) of the lewisite to the rabbits. However, in both rat and rabbit studies, changes in maternal body weight measures, which were evident only in treatment groups in which mortality occurred, did not appear to be sensitive indicators of lewisite toxicity. Also, significant fetal effects, which were limited to signs of retarded development, were observed only at dose levels that induced maternal mortality. These results suggest that maternal mortality was the most important factor in predicting the induction of maternal and fetal effects and, therefore, a "no observable effect level" in maternal animals and their fetuses would be between 1.5 and 2.0 mg/kg in rats and less than 0.07 mg/kg in rabbits.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 85-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the product or services of these organizations.

TABLE OF CONTENTS

APPENDICES
(See Final Report, Part 2, dated December 31, 1987.)

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

OBJECTIVE

To determine the potential of lewisite for the induction of teratogenic or toxic effects in fetuses of CD rats and NZW rabbits following daily, intragastric administration of the agent to maternal animals during the period of gestation that encompasses major organogenesis.

SPECIFIC AIMS

1. To determine significant toxic response to lewisite in pregnant rats and rabbits as measured by mortality and alterations in body weight parameters.

2. To identify and determine significant effects (increased incidences of intrauterine mortality, retarded fetal development and fetal malformations) in the conceptuses of animals exposed to lewisite during gestation.

BACKGROUND

For lewisite, a highly toxic chemical that produces severe skin burns on contact, there is a potential for occupational exposures in research or industrial laboratories. Exposures of the general population would more likely result from accidental release of this agent during transport or in an industrial accident. Segments of the general population, which include the chronically ill, the young and old, and pregnant women, have been identified as populations that are particularly sensitive to most toxic agents. It is this concern that has promoted studies to identify potentially toxic and teratogenic agents and to develop a data base for the establishment of hazard evaluations and occupational health standards for these chemicals.

Dichloro(2-chlorovinyl)arsine (lewisite) and sulfur mustard are prototypes of two categories of vesicant war gases. Unlike the strong alkylating agent, sulfur mustard, lewisite reacts with the sulfhydryl groups of proteins through its arsenic group (Cassarett and Doull, 1986).

During World War I, several arsenicals were utilized as chemical warfare agents and, although lewisite was developed during this period, it was never used in the field until 1938 at Ichang. More recently, London physicians, who treated victims of chemical weapons in the 1984 Persian Gulf War, observed typical symptoms of the effect of this agent (Perera, 1985). Early in World War II, interest in the toxicology of chemical warfare agents revived and many of the animal studies with lewisite were performed during the 1940's (Windholz, 1983). During this interval, therapeutic agents for treatment of lewisite exposures were studied and British anti-lewisite (BAL) was developed (Peters et al. 1945). BAL, a vicinal dithiol with a greater affinity for trivalent arsenic than for protein, has also been used to treat heavy metal poisoning. More recent studies of the lethal systemic action of lewisite have also been concerned with the development of therapeutic agents

using chemical analogs of BAL, dimercaptosuccinic acid and dimercaptopropanesulfonate (Aposhian, 1982; Hsu et al., 1982).

In 1946, a comprehensive review of the production and the chemical, physical and physiologic properties of lewisite was prepared by the National Defense Research Committee (Gates, et al. 1946). This review summarized toxicologic data of lewisite acquired during World War I and the early 1940's, and compared human and animal data as well as the effects of exposures to lewisite and sulfur mustard. Although this review concluded that, by the end of World War II, sufficient toxicologic data were available for the determination of dosages for military purposes, certain discrepancies in the lewisite dose levels required to induce skin lesions and systemic intoxication should be resolved. Many of the studies performed during this period demonstrated a biological basis, namely species' sensitivity, for these differences, but we may speculate that certain properties of the lewisite preparations used in these toxicologic studies may have contributed to the variability in the results. These properties included the presence of impurities in earlier preparations, the volatility of the chemical and its instability in the presence of moisture, and differences in relative amounts of the two isomers of lewisite. In the presence of water or alkalies, lewisite hydrolyzes to form lewisite oxide, which is non-volatile and insoluble in water. Lewisite oxide is apparently a much weaker vesicant than lewisite (Gates, Williams and Zapp, 1946) and, because of its greater insolubility in aqueous medium, might be expected to induce less systemic toxicity. No references to studies of lewisite oxide toxicity were found in the literature and only one study concerning the toxicity of the two lewisite isomers was cited. In this study, mice were exposed to vapors of chorarsine derivatives, including the two isomers of lewisite, and the isomers were found to be equally toxic (OSRD 823, cited in Gates, Williams and Zapp, 1946).

Exposure to lewisite vapor produces edema of the respiratory tract and accumulation of pleural fluid (U.S. War Department, Technical Manual 8-285, cited in Gates, et al., 1946). Skin lesions resulting from contact with liquid lewisite involve the rapid formation of an erythematous area, subsequent vesication and penetration of subcutaneous tissue so that edema and necrosis are evident. Species' sensitivity to skin lesion induction was rabbit >dog >man. Systemic intoxication, as studied in dogs (U.S. Report Ph. #237 cited in Gates, et al., 1946), was evident a few hours following application of the lewisite. This report stated that "apparent interference with certain vital processes did not produce sufficient anatomical lesions to characterize the immediate cause of death"; however, these workers found that fluid losses due to changes in capillary permeability caused remarkable decreases in blood volume. Comparisons of toxic effects of lewisite and sulfur mustard in dogs and rabbits indicated that lewisite was more damaging to the skin and was more likely to induce systemic poisoning than was sulfur mustard.

It is of interest to note that many of the symptoms of arsenic and lewisite intoxication are similar: severe inflammation of the gastrointestinal tract with electrolyte disturbances and ulceration and perforation of membranes (NAS, 1977). In alkaline solutions, lewisite hydrolyzes to form acetylene and sodium arsenate. Arsenic, as sodium arsenate or arsenite, is

known to be embryotoxic and teratogenic in a number of animal species (Leonard and Lauwerys, 1980). In rats, a single, intraperitoneal (IP) dose of 30 mg of arsenate/kg, administered on 9, 10, or 11 days of gestation, was teratogenic (Beaudoin, 1974). In mice, an IP dose of 45 mg/kg, administered once between 6 and 12 dg, increased prenatal mortality and the incidence of fetal malformations and decreased fetal growth, but a dose level of 25 mg/kg, delivered daily from 6 through 12 dg, did not induce fetal effects that were significantly different from control values (Hood and Bishop, 1972). These workers also found that oral doses of 120 mg/kg were required to cause maternal toxicity and a decrease in fetal body weights. Because of these studies, and others such as those of Ferm and Carpenter (1968) in the hamster, it is generally accepted that arsenic in its anionic form is able to cross the placental membranes. Early studies, which have been reviewed by Leonard and Lawerys (1980), showed that organic arsenicals, including those used in human medicine, were stored in the placenta of the cat, rabbit and human but apparently did not cross the placental membranes readily.

The accumulation of a sufficient quantity of arsenate to induce a teratogenic effect following oral administrations of lewisite for 10 or 14 days, would be influenced by a number of factors. First, the value of an LD_{50} for a single dose of lewisite, which contains 36% arsenic, in the rat is 50 mg/kg (Table 1) and this dose level would not be proposed for a study which involves repeated, daily administration of the agent. The chemical form of arsenic governs its absorption from the gastrointestinal tract, which is usually in excess of 50% of the amount ingested, however, about 50% of the absorbed arsenic is eliminated within 10 days (Dutkiewicz, 1977). Furthermore, intragastric administration of the lewisite would deliver the agent into an acid environment in which the hydrolysis products would be chlorovinyl arsenous oxide and HCl, and not sodium arsenate. Although the metabolism of chlorovinyl arsenous oxide has evidently not been studied, we may speculate that, under these chemical and metabolic restraints, the accumulation of toxic levels of arsenic in maternal animals and their fetuses would not be expected in a relatively short-term study of 10 to 14 days.

A search of the open literature did not reveal any information concerning the teratogenic, mutagenic or carcinogenic properties of lewisite. Indirect evidence of the potential carcinogenicity of lewisite may be inferred from a paper by Krause and Grussendorf (1979) who reported that a skin carcinoma occurred in the cicatricial area 39 years after lewisite contamination.

STUDIES AT PACIFIC NORTHWEST LABORATORY

Studies to determine the effects of lewisite administration during the period of major organogenesis in rats and rabbits required information concerning the toxicity of lewisite in pregnant animals following multiple intragastric exposures (10 or 14 consecutive daily doses for the rat and rabbit, respectively.) Information available from the literature provided one value, an LD_{50} for a single oral dose of lewisite in the rat (Table 1). Therefore, it was necessary to conduct preliminary toxicity studies to determine the amount of residual injury to maternal animals and fetuses of both species following short-term multiple exposures. In each case, the

preliminary dose-range studies included pregnant animals dosed with the vehicle or one of four dose levels of lewisite. Subsequent teratology studies involved the study of groups of animals dosed with the vehicle or one of three levels of the agent.

TABLE 1. LD_{50} Values* for Lewisite

Route of Administration	LD_{50} Value (mg/kg)	
	Rat	Rabbit
Intravenous	---	0.5
Subcutaneous	1	2
Dermal	24	6
Oral	50	---

*Registry of Toxic Effects of Chemical Substances, 1980 and 1983.

MATERIALS AND METHODS

LEWISITE

Procurement and Characterization

A shipment of 25 ml of dichloro(2-chlorovinyl)arsine (lewisite, Agent L) was received from the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) on 7 March 1985. Relevant chemical and physical properties of this agent are listed in Table 2. The chemical (Lot No. L-U-4273-CTF-N) was prepared by distillation on 30 September 1984 at the Chemical Research and Development Center (CRDC). The agent was analyzed by nuclear magnetic resonance (H-1 and C-13; CRDC SOP No. 6-1-83-1, Annex F) at the Research Directorate, CRDC. Results of the analyses, expressed as calculated weight percent, were 95.8 and 4.0 for trans and cis isomers of dichloro(2-chlorovinyl)arsine, respectively, and 0.2 for unknown compounds.

The lewisite was divided into two equal portions, pipetted into 30-ml Wheaton vials, sealed and stored in secondary unbreakable containers in refrigerated storage at ~6°C. Lewisite solutions for all four studies were

TABLE 2. Relevant Chemical and Physical Data for Lewisite, Dichloro(2-Chlorovinyl)arsine*

CAS #:	541-25-3
RTECS #:	CH2975000
Structural formula:	$Cl\text{-}CH{=}CH\text{-}AsCl_2$
Molecular weight:	207.3 g
Density at 20°C:	1.888 g/ml
State:	Dark, oily liquid (stable in steel and glass)
Vapor pressure at 20°C:	0.394 mm
Decomposition temperature:	>100°C
Solubility in water:	Very slightly soluble
Hydrolysis	
Rate:	Rapid
Products:	Chlorovinyl arsenous oxide, HCl (in acid solutions)
	Acetylene, sodium arsenate (in alkaline solutions)

*Obtained from USABRDL, Rosenblatt et al. 1975

prepared from one (CSM-2-85) of the two vials. To comply with Good Laboratory Practices requirements, PNL has requested that USAMRICD retain an aliquot of this lot of lewisite.

After the studies had been completed, lewisite from lot CSM-2-85 was analyzed to detect the presence of common impurities, such as lewisite oxide and the cis-trans isomers of bis(2-chlorovinyl)chloroarsine and tris(2-chlorovinyl)arsine (Rosenblatt et al., 1975). Measurement of the ultraviolet absorption spectrum of the sample in isooctane revealed that the spectrum and the absorptivity of the material at 215 nm agreed with published values in the literature (Rewick, et al., 1986; Mohler and Sorge, 1939) and did not indicate the presence of ultraviolet-absorbing compounds other than lewisite. This conclusion was supported by results from gas-chromatographic analyses of the sample following derivatization with 2-mercaptoethanol (Appendix A).

Characterization of the Diluent

The selection of a satisfactory vehicle to use in preparing lewisite solutions for administration to the animals was based on the chemical and physical properties of the compound, i.e., its relative insolubility and rapid hydrolysis in water. Corn oil is commonly the vehicle of choice for the administration of water-insoluble compounds, but several reports have indicated that the use of corn oil may not be appropriate for reproduction studies. Corn oil was reported to induce uterine hypertrophy (Duncan, G., 1984, Personal Communication), and to alter immune function in developing rats (Springer, 1982) and mice (Shepman and Schmidt, 1984). A recent comparison (Kimmel et al., 1985) of reproductive measures in rats dosed with distilled water or corn oil indicated that fetal body weights were lower and that the incidence of malformations was higher in litters of dams dosed with corn oil from 6 through 15 dg. In considering an alternative to corn oil, we found that sesame oil was classified as "generally recognized as safe" by the Food and Drug Administration (FDA; Furia, 1972) and that the major fatty acid components were quite similar in both oils (Altman and Dittmer, 1972); however, sesame oil contains a lower concentrations of sterols (0.49%) than corn oil (0.79%). Sesame oil is readily available, contains no preservatives and appears to be quite stable when stored under the proper conditions; however no historical data for reproductive or developmental effects are available in the literature.

For these studies, four lots of sesame oil, produced by the Hain Pure Food Company (Los Angeles, CA) were purchased locally. An effort was made to obtain bottles from a single lot; however the presumptive lot number on the bottles, B11421/B (a), did not change with time and apparently was not a lot number. Subsequent lots were identified by notebook number (Table 3).

Individual bottles of sesame oil used for the studies were analyzed to determine their peroxide content, which serves as a measure of oxidation or rancidity of the oil. The method used quantifies peroxides (and similar substances) that oxidize aqueous iodide under the conditions of the test. The iodine produced is determined potentiometrically by titration with sodium thiosulfate. Each batch of sesame oil was analyzed prior to its use for the

TABLE 3. Analyses of Sesame Oil for Peroxides

Lewisite Study	Lot Number	Date of Purchase	Date of Analysis	Container Identification	Peroxide[a] Content (meq/L)	PNL Notebook Reference
Rat, Dose-Range	B11421/B(a)	10/1/84	3/11/85	E	0.44	50113/39-43
Rat, Teratology	50775-29	1/15/86	2/19/86	7	5.7	51337/31-35
Rabbit, Dose-Range	50775-82	6/12/86	6/12/86	1	5.7	51563/7-9
			6/12/86	2	5.6	51563/9-12
Rabbit, Teratology	50775-95	7/11/86	8/20/86	8	6.0	51563/20-22
				11	6.0	51563/25-27

[a]Analyses performed in accordance with the standard operating procedures ØB-AC-3AØR (Analysis of Sesame Oil for Peroxide) and ØB-AC-3B1D (Standardization of Dilute Sodium Thiosulfate).

dilution of the lewisite solutions. All analyzed values were within acceptable limits for peroxides (10 meq/L), which had been established in previous studies at PNL.

Preparation of Solutions for Administration

The dilution procedure for the preparation of the dosing solutions was performed according to SOP ØB-AC-3B18 (Dilution of Neat CSM). The solutions were prepared in two lots, one before the initiation of dosing and one at about the midpoint of the dosing interval. Once the required volume of the agent and the final concentrations had been determined, the volume was removed from the bottle of neat lewisite and thoroughly mixed in a known volume of sesame oil. Aliquots of this intermediate concentration were then diluted further to give the final concentrations needed for the dosing solutions. Once the final solutions were prepared, aliquots of the solution were placed in Wheaton bottles with teflon-lined septa and aluminum caps. Each Wheaton bottle contained a sufficient volume of lewisite/sesame oil for 1 day's use. The bottles were labeled with the name of the agent and the concentration. The bottles were then placed in a second unbreakable container that was labeled with the following information: solution identification, lot number, concentration, date, PNL Laboratory Notebook number, and initials of preparer. The bottles were then stored in a refrigerated storage container at ~6°C until used.

Analyses of Lewisite Solutions

Lewisite in sesame oil was assayed by gas chromatography, using a capillary column and flame-ionization detection. Substantial analytical problems were encountered during the first two studies (dose-range and teratology studies in rats), and the procedure was subsequently modified for analyses of the solutions used in the rabbit studies. The presence of some high-boiling components in the sesame oil required that the temperature of the capillary-column inlet be maintained at 200°C. Since the decomposition temperature for lewisite is low (190°C), it was necessary to develop an assay that would permit the migration of sesame oil through the column without any decomposition of the lewisite. To solve this problem, a stable derivative of lewisite in sesame oil was prepared by the addition of 2-mercaptoethanol. The reaction, which proceeds at room temperature, may be written:

$$ClCH{=}CHAsCL_2 + 2\ HSCH_2CH_2OH \longrightarrow ClCH{=}CHAs(SCH_2CH_2OH) + 2\ HCl$$

In the procedure developed for the analysis, lewisite samples with concentrations $\leq$2.0 mg/ml were diluted 1:10 with isooctane prior to analysis. For the assay, 0.5 ml of the sample was diluted with 0.5 ml of a solution containing 120 ng of 1-chloronaphthalene and 5584 ng of 2-mercaptoethanol/μl in isooctane contained in a 1.5 ml automatic sampler vial with a Teflon-lined, crimped-top cap. The column (J&W DB-5) temperature program was 80°C for 5 min (5°/min) to 140°C, 20°/min to 300°C and 300°C for 20 min. A Hewlett-Packard 5840A gas chromatograph and a 7672A automatic sample changer were used (Appendix A).

Results of the analyses for lewisite in the solutions prepared for each study are shown in Table 4. No results were obtained for solutions used in the rat dose-range study and the first lot of solutions for the rat teratology study. The analyses of the second lot of solutions for the rat teratology study, using a low column temperature and no derivatization of the lewisite, were not sufficiently sensitive to detect concentrations of lewisite below 0.45 mg/ml. Solutions for the rabbit dose range study were diluted 1:10 with isooctane and each lot was analyzed at the time of preparation as well as 7 to 11 days later. The results were within acceptable limits of analytical error, except for those analyzed 7 days after the preparation of the first lot. These values were 137-147% of the target concentrations and were probably due to analytical error. Values for the solutions from the rabbit teratology study were consistent, although the results from the 0.8 mg/ml solution were somewhat high (114-124%) and the 0.28 mg/ml were very low (39-64%). The occurrence of low values suggested rapid degradation of lewisite in the sesame oil, possibly from the presence of water in the oil, or a loss in sensitivity of the method at lower concentrations. However, the facts that the analytical value for the first lot, obtained 6 days after preparation of the solution, was consistent with the value obtained at the time of preparation and, also, that the target concentration of 0.28 mg/ml does not differ substantially from the assay concentration of the solution in the rabbit dose range study (2.0 mg/ml diluted 1:10 with isooctane), suggest that a probable cause for the low analytical values in the teratology study may be interference by some constituent of the sesame oil.

TABLE 4. Analyses of Lewisite Solutions Used for Animal Studies

Study	Date of Preparation	Date of Analysis	Dose Level (mg/kg)	Lewisite Concentration (mg/ml) Calculated	Lewisite Concentration (mg/ml) Analyzed[a]
Rat Teratology	2/27/86	3/7/86	0.5	0.15	--
			1.0	0.30	--
			1.5	0.45	0.47 ± 0.02
Rabbit, dose-range	6/13/86	6/13/86	0.5	2.0	2.09 ± 0.12
			1.0	4.0	4.26 ± 0.02
			1.5	6.0	6.00 ± 0.19
			2.0	8.0	8.02 ± 0.02
		6/20/86	0.5	2.0	2.73 ± 0.26
			1.0	4.0	5.86 ± 0.01
			1.5	6.0	8.71 ± 0.32
			2.0	8.0	11.00 ± 0.04
	6/20/86	6/20/86	0.5	2.0	1.97 ± 0.06
			1.0	4.0	4.34 ± 0.03
			1.5	6.0	6.16 ± 0.05
			2.0	8.0	7.70 ± 0.30
		7/1/86	0.5	2.0	2.12 ± 0.10
			1.0	4.0	3.88 ± 0.17
			1.5	6.0	5.81 ± 0.30
			2.0	8.0	8.29 ± 0.42
Rabbit Teratology	8/22/86	8/22/86	0.07	0.28	0.14 ± 0.02
			0.2	0.8	0.94 ± 0.03
			0.6	2.4	2.39 ± 0.01
		8/28/86	0.07	0.28	0.18 ± 0.02
			0.2	0.8	0.94 ± 0.03
			0.6	2.4	2.33 ± 0.28
	8/28/86	8/28/86	0.07	0.28	0.22 ± 0.04
			0.2	0.8	0.95 ± 0.01
			0.6	2.4	2.29 ± 0.12
		9/8/86	0.07	0.28	0.11 ± 0.01
			0.2	0.8	0.99 ± 0.03
			0.6	2.4	2.60 ± 0.05

[a]Mean ± SE of duplicate samples (n = 2).

ANIMAL MAINTENANCE

Procedures for Rats

Young, adult CD female (7 to 8 weeks old, 170 to 175 g) and male (7 to 8 weeks old, 200 to 225 g) rats of Sprague-Dawley derivation were obtained from Charles River Breeding Laboratories, Inc., Portage, MI Facility (dose-range study) and the Raleigh, NC Facility (teratology study). Groups of 108 females and 58 males (for the dose-range study) and 211 females and 117 males (for the teratology study) were purchased to provide sufficient pregnant animals for the studies, to provide additional animals for evaluation of health status, and to compensate for any losses in shipment.

Upon arrival at PNL, the rats were placed in an isolated facility and remained within this facility until sacrifice. Specified environmental conditions for animal rooms were temperatures of 72 ± 3°F, relative humidities (RH) of 50 ± 15% and a lighting cycle of 12 hours on/12 hours off. During quarantine, the animals were group-housed in stainless-steel, wire-mesh cages placed in automatic-flush racks provided with an automatic water system. Females in which sperm were detected were housed in individual cages in similar cage racks. Purina Certified Rodent Chow (#5002) and water were provided ad libitum.

Purchase requisitions specified that the rats were to be free of Mycoplasma, Sendai virus and Corynebacterium. Upon receipt, six rats (one female and five males) from the shipment for the dose-range study were removed for health evaluations. Their sera were tested for antibodies to Sendai virus, pneumonia virus of mice (PCM), rat corona virus/sialodacryoadenitis virus (RCV/SDA), H-1 virus and Kilham rat virus (KRV) by Microbiological Associates (Bethesda, MD). Ten rats (five females and five males) from the shipment for the teratology study were tested for Sendai virus, PVM, RCV/SDA and M. pulmonis by serological methods in our laboratory. Additional evaluations included cultures of nasopharyngeal and lung washes; examination of animals for internal and external parasites; and histopathologic examination of lungs, trachea, Harderian gland, heart, liver, kidney, ileum and colon. No significant pathogens or pathologic lesions were detected in rats of either shipment.

Following an isolation period of 3 weeks, the females were individually identified by means of a numbered eartag and weighed. The rats were allowed to mate overnight by caging one male with one female. Copulation was established by detecting the presence of sperm in the vagina, as determined by microscopic examination of a slide prepared from a lavage suspension of normal saline delivered into, then recovered from, the vagina with a pipette. Mating was continued nightly until sufficient sperm-positive rats were obtained for the study. The morning of observation of sperm was designated as 0 dg. At that time, females that had mated the previous night were weighed and assigned to treatment groups by means of formal randomization, blocking on weight and using a statistical software package. Each treatment group of rats was identified by an appropriate toe clip, in addition to the individual identification by numbered eartags. Cage cards for individual rats indicated the animal number, treatment group and gestational group.

Procedures for Rabbits

Sexually mature, New Zealand White rabbit does (5 to 6 months of age; body weight about 3 kg) were obtained from R & R Rabbitry (Stanwood, WA). In addition to the 114 does used in the studies, 7 additional does were obtained to be used for training bucks to the artificial vagina (AV), or for replacements to compensate for shipping losses or in the event that the rabbits' health status might interfere with the study results. Six mature, naive bucks (6 to 7 months old; body weight 3 to 4 kg) of the same stock were purchased for breeding in the dose-range study; an additional six bucks were purchased for the teratology study. All rabbits were identified by the supplier with a uniquely numbered, stainless-steel eartag.

Upon arrival at PNL, the rabbits were placed in an isolated facility, where they remained until sacrifice. Specified environmental conditions for animal rooms were temperatures of 70 ± 4°F, RH of 50 ± 15% and a lighting cycle of 16 hours on/8 hours off. The animals were housed individually in stainless-steel, wire-mesh cages in automatic-flush racks. They were provided with Purina Certified Rabbit Chow (#5322) and water ad libitum. Food consumption was not measured, but estimates of the amount eaten by each animal were obtained by observing the feeders, which were completely filled with food each day. The rabbits were considered to be anorectic if their food, which had been leveled at the top and bottom of the feeder, had not been disturbed.

To obviate the possibility of pseudopregnancy and to permit frequent observations of their health status, the does were maintained for 21 days (dose-range study) and 24 days (teratology study) before use. For the first 10 days of this period, oxytetracycline was added to the drinking water for prophylaxis. The rabbits were examined by the PNL Clinical Veterinarian and were found to be acceptable for these studies.

Following quarantine and acclimation, the does were weighed and assigned to treatment groups by means of a formal computerized-randomization program. Artificial insemination (AI) was performed in the afternoon of the day designated as 0 dg.

Bucks to be used as semen donors were trained to service an AV during a 2- to 3-week period (Gregoire et al., 1958). On the day of AI, semen samples from at least 3 bucks were collected into an AV equipped with a reservoir warmed to 42 to 45°C just prior to use (Adams, 1961; Hafez, 1970; Tesh and Tesh, 1971; Hagen, 1974). The individual samples were evaluated for motility, sperm concentration and the presence of urine, bacteria, erythrocytes and leukocytes. Semen samples of satisfactory quality were pooled and diluted with buffered citrate/egg-yolk extender to a concentration of 21 to 30 million sperm/ml (Table 5).

The does were inseminated with approximately 0.5 ml of the extended semen within 2 hours of semen collection. To induce ovulation, 100 USP units of chorionic gonadotropin (Ayerst, 500 USP units/ml in saline) were administered to each doe by IV injection immediately after insemination.

TABLE 5. Characteristics of Extended Rabbit Seminal Fluids Used for Artificial Insemination[a]

Study	Day of Insemination	Number of Does Inseminated	Number of Donor Bucks	Sperm Characteristics		
				Concentration (10^6/ml)	Motility	
					Grade[b]	Percent
Dose Range	1	20	4	22	3	80
	2	20	4	21	3	83
Teratology	1	24	4	26	4	91
	2	25	4	30	4	87
	3	25	4	21	4	88

[a]Pooled semen samples diluted with buffered citrate/egg-yolk extender
[b]Graded from 0 (no motility) to 4 (excellent motility)

ADMINISTRATION OF LEWISITE

Solutions of appropriate concentrations of lewisite in sesame oil were administered to the animals by IG intubation, in the morning, on consecutive days. Rats were dosed for 10 days (6 through 15 dg); rabbits were dosed for 14 days (6 through 19 dg). Daily doses for individual animals were calculated from their body weight, which was determined just prior to dosing. The dosage volume/body weight was 1 ml/300 g for rats and 1 ml/4 kg of body weight for rabbits.

The dosing solution was measured with a syringe and delivered to the rats with an 18-ga, 3-in. feeding needle terminating in a 2.25-mm ball. For rabbits, a #8 French 22-in. feeding tube was used to ensure delivery of the dose solution into the stomach. To minimize the possibility of injury to the animal during dosing, the rats were restrained by the technician who delivered the dose. Rabbits were restrained by enclosure in a canvas bag, which was hand-held in a plastic box by an assistant to the technician who delivered the dose.

TOXICOLOGIC AND DEVELOPMENTAL EVALUATIONS

All animals were observed for clinical signs of toxicity in the morning prior to, and following, the administration of the lewisite, and observed again in the afternoon. The condition of the animals was observed twice daily on experimental days on which no chemical was administered. Body weights of rats were determined prior to mating and on 0, 6 through 16, and

20 dg; body weights of rabbits were measured prior to AI and on 0, 6 through 20, and 30 dg.

Necropsies were performed on animals found dead and those that were euthanized because they were judged too moribund to complete the scheduled experimental regimen. All gross observations for abnormalities were recorded and grossly abnormal tissues were preserved in 10% neutral buffered formalin (NBF). Ovaries were removed, and the corpora lutea were counted; the uterus was opened and examined for the number and position of viable fetoplacental units and of resorption sites.

At scheduled sacrifice (20 dg for rats and 30 dg for rabbits), the animals were killed in a randomly determined order by introduction of carbon dioxide into a euthanasia chamber. The animals were identified only by their unique identification number to assure that the treatment group was not known to the prosectors. Foot markings of the rats were observed only by the prosectors performing the necropsy of the adult animals, so that the exposure group was not known to those evaluating reproductive status and fetal measures. Body weights and uterine weights were measured and recorded. All animals were examined for evidence of infections or lesions. Grossly abnormal tissues were preserved in 10% NBF.

The uterus, with ovaries attached, was removed from each animal and weighed. The ovaries were excised, identified as to right and left, and the number of corpora lutea estimated by counting. Uteri of all apparently nonpregnant females were stained with ammonium sulfide and examined for implantation sites (Kopf et al., 1964). In pregnant animals, the excised uterus was opened. Beginning at the right ovary, numbers were assigned consecutively to each implantation site down the right horn to the cervix. Consecutive numbers for implantation sites in the left horn proceeded from ovary to cervix. The membranes and amniotic fluid were observed for abnormalities, and living and dead fetuses and resorptions were counted. For the rat, mortality in utero was classified as "early" (placenta and conceptus indistinguishable, or metrial gland), "mid" (placenta distinct, embryo partially to fully formed), "late" (fully formed with evidence of resorption), or "dead" (no movement detected at necropsy). For rabbits, mortality in utero was classified and recorded as "early" (placenta and conceptus indistinguishable, or metrial gland), "late" (placenta distinct, embryo or fetus partially to fully formed), or "dead" (no movement detected at necropsy).

For dose-range and teratology studies, fetal observations and measurements included position in the uterus, viability, weight and gross observations for morphologic defects. Placentas were examined but were not weighed for the dose-range studies.

Live and recently dead fetuses were removed in serial order, freed of adherent material and weighed. Each fetus was examined for gross external abnormalities. The sex of each rat fetus was determined by external inspection of the anogenital distance; the sex of rabbit fetuses was determined by internal examination because of the variability in anogenital distance encountered in this species (Njielsen and Torday, 1983).

For teratology studies, the crown-rump length of each fetus was measured. Concurrently, the placentas were removed, weighed and examined; abnormal placentas were preserved in 10% NBF. Fetuses of both species were randomly divided into two equal groups for more detailed teratologic examination. Complete skeletons were examined in one group, in the second group, the heads were removed and placed in Bouin's fixative for subsequent examination of serial razor-blade-cut sections by the methods of Wilson and Warkany (1965) and van Julsingha and Bennett (1977) for rats and rabbits, respectively.

All fetuses in the teratology studies were examined for internal abnormalities by dissection, using Staples' (1974) technique, which is a modification of that of Barrow and Taylor (1969), and is similar to those described by Stertz (1977) and Stuckhardt and Poppe (1984). The sex of each fetus was determined by visceral examination of the gonads. All fetuses were eviscerated; rat fetuses were skinned and immediately fixed in alcohol; rabbit fetuses were skinned and air-dried prior to fixation in alcohol. Following staining with Alizarin red S, maceration with KOH and clearing in glycerol (Staples and Schnell, 1964), all components of each skeleton were examined for abnormalities in size, shape, relative position and degree of ossification. Results from fetal morphologic examinations were grouped into three categories (major malformations, minor abnormalities or morphologic variations) according to degree of severity, locus of fetal structural change and incidence of these changes (Palmer, 1977, 1978; Perraud, 1976).

Data for body weights of adult animals are from pregnant animals only. Although formal randomization of body weights was used to select animals for the experimental groups, the fact that data from pregnant animals only were analyzed tended to produce apparent deviations in initial body-weight values for some groups of animals. Extragestational body weight (body weight at necropsy minus weight of the gravid uterus) and extra-gestational gain (extragestational weight minus body weight on 0 dg) were calculated for each maternal animal.

STATISTICAL METHODS

The computer software program (DRANDBLK) for randomizing animals into experimental groups is based on a single blocking factor, animal weight at 0 dg. Animal weights for a given study are ordered from lightest to heaviest; blocks of animal weights were then randomly assigned to the treatment groups and the control group. Block sizes were governed by the number of test groups. Probit analyses of lethality were conducted using a computer software program (SAS, 1985).

Analysis of variance, which was used to analyze continuous variable data, employed the General Linear Model (GLM) procedure in the Statistical Analysis System (SAS, 1985). If the F statistic was significant, Duncan's (1955) multiple range test was used to delineate intergroup differences. A comparisonwise error rate was set at 0.05 for Duncan's test. By using this comparisonwise error rate in the Duncan's multiple range test, more significant differences may be detected than would result from the use of a multiple comparison procedure that sets the experimentwise error at 0.05. Data were

also compared by orthogonal contrasts to determine if there was a trend with respect to increasing dose.

A randomization test was used to analyze body-weight measurements. This test is a nonparametric statistical test that is based on the absolute area between growth curves. The test allows for correlation of body-weight measurements over time (Zerbe, 1979).

Fetal body weight and crown-rump lengths for live fetuses were analyzed by nested analysis of variance. The analysis takes into account the effects of treatment, litter and sex on the body weight and crown-rump length measurements.

Pairwise comparison of binary response variables among groups was done by chi-square tests or Fisher's Exact Test (Siegel, 1965) using the P4F program in the BMDP statistical software (Dixon et al., 1983). Chi-square tests were used for pairwise comparisons for fetal data, and Fisher's Exact Test was used for litter data, where a litter with one or more fetuses responding was considered a positive litter response.

DOSE-RANGE AND TERATOLOGY STUDIES IN RATS

One hundred eight adult female rats were mated for 4 consecutive nights to provide 51 sperm-positive animals for the dose-range study. These animals were weighed and assigned to treatments groups that received the following dose levels of lewisite: 0 (vehicle control), 0.5, 1.0, 2.0 and 2.5 mg/kg (Table 6).

TABLE 6. Experimental Design for Lewisite Studies with Rats

Dose Level (mg/kg)	Number of Sperm-Positive Females	
	Dose-Range Study	Teratology Study
0	10	25
0.5	10	25
1.0	10	25
1.5	--	25
2.0	10	--
2.5	11	--

For the teratology study, 211 adult female rats were mated for 4 consecutive nights to obtain the 100 sperm-positive females used in this study. The rats were weighed and assigned to treatment groups by formal randomization. Data from the dose-range study was used to establish the

following dose levels: 0 (vehicle control), 0.5, 1.0 and 1.5 mg lewisite/kg (Table 6).

Lewisite solutions were administered by IG intubation in the morning on consecutive days from 6 through 15 dg. The animals were weighed prior to mating and on 0, 6 through 16, and 20 dg and observed for behavior and signs of toxicity at least twice daily. At scheduled sacrifice (20 dg), maternal animals were weighed and examined for gross abnormalities and reproductive status. Procedures for these observations and for the fetal evaluations for each study have been detailed in previous sections.

DOSE-RANGE AND TERATOLOGY STUDIES IN RABBITS

For the dose-range study, 40 mature New Zealand White rabbit does were artificially inseminated on 2 consecutive days. On each day, the does were randomly distributed into five treatment groups on the basis of their weight on 0 dg. Dose levels of lewisite for this study were 0 (vehicle control), 0.5, 1.0, 1.5 and 2.0 mg/kg (Table 7).

In the teratology study, 74 females were artificially inseminated on 3 consecutive days (24, 25 and 25 does on days 1, 2 and 3, respectively). The does were randomly assigned to four dose groups: 0 (vehicle control, 0.07, 0.2 and 0.6 mg/kg (Table 7). The selection of these dose levels was based on information obtained in the dose-range study.

TABLE 7. Experimental Design for Lewisite Studies with Rabbits

Dose Level (mg/kg)	Number of Sperm-Positive Does	
	Dose-Range Study	Teratology Study
0	8	19
0.07	--	18
0.2	--	18
0.5	8	--
0.6	--	19
1.0	8	--
1.5	8	--
2.0	8	--

The lewisite solutions were administered to the rabbits by IG intubation in the morning on consecutive days from 6 through 19 dg. The animals were weighed prior to AI and on 0, 6 through 20, and 30 dg and observed for clinical signs of toxicity at least twice daily. At scheduled sacrifice (30 dg), maternal animals were weighed and examined for gross abnormalities and

reproductive status. Details of these procedures and for fetal evaluations for each study appear in previous sections.

SAFETY PROCEDURES AND GOOD LABORATORY PRACTICES

Personnel involved in laboratory and animal manipulations for these studies with lewisite complied with procedures listed in the document, "Facility Security and Safety Plan for Neat Vesicants and Dilute Organophosphates", which was prepared for the Department of the Army, U.S. Army Medical Research Institute of Chemical Defense by the Pacific Northwest Laboratory prior to the initiation of the studies and was revised in March 1986.

All facets of these studies were conducted in compliance with the Good Laboratory Practice Regulations for Nonclinical Studies, 21 Code of Federal Regulations, Part 58.

RESULTS

DOSE-RANGE STUDY IN RATS

The status of the rats in the dose-range study is shown in Table 8 and the observations at necropsy are summarized in Table B1 of the Appendix. In animals whose death (at 12 to 16 dg) was attributed to lewisite toxicity, an extremely flatulent gastrointestinal tract (GIT), partially filled with yellow and bloody fluid, was commonly observed at necropsy. In some instances, backflow of the dosing solution had been noted in these rats at the time the dose was administered, but in each case no gross abnormalities were observed in the respiratory tract, esophagus or thorax at necropsy. Except for one rat of the 2.0 mg/kg dose group, the characteristic appearance of the lewisite-exposed GIT was evident in all animals that died from dosing injury, including one rat in the 1.0 mg/kg dose group. At scheduled sacrifice,

TABLE 8. Status of Rats in the Dose-Range Study of Lewisite

Observations	Dose Level (mg/kg) 0	0.5	1.0	2.0	2.5
Numbers of rats/ group	10	10	10	10	11
Number of rats:					
With no CL[a]	3[b]	1	1	1	2
With CL/no implants	0	0	1	0	1
Not pregnant	2	1	2	1	3
Pregnant	8	9	8	9	8
Number of survivors	10[b]	10	9	7	8
Pregnant	8	9	8	6	5
Not pregnant	2	1	1	1	3
Probable cause of death[c]					
Lewisite toxicity	0	0	0	1	2
Dosing trauma	0	0	1	2	1

[a]Corpora lutea (CL).
[b]Implantation site detected in rat 356 by uterine stain; no CL evident. Data from this animal and rat 331, in which a perforated lung was observed at necropsy, were omitted from all summaries.
[c]Observations and findings at necropsy are listed in Table B1 of Appendix B.

a small gastric ulcer was noted in an animal of the highest dose group and two lung lesions were observed, one in this group and one in the control group.

Although a significant linear trend in reduced maternal body weights with increasing dose levels was observed by the end of dosing (15 and 16 dg), no significant differences among treatment groups for body weights of pregnant survivors were evident until sacrifice (Table 9). At this time, body weights of rats in the highest dose group (2.5 mg/kg) were significantly decreased and values for animals dosed with 2.0 mg/kg tended to be lower than the controls. From 12 to 15 dg, values for rats in the 2.0 mg/kg dose group tended to be lower than all other treatments and may have reflected the mid-gestational death of embryos, which occurred in two of the six rats in this group (Table C1 in Appendix C). Extragestational body weights and extragestational gains (Table 10) tended to be decreased in the rats that received more than 0 5 mg/kg of lewisite and were significantly decreased in the 2.5 mg/kg dose group compared to controls. Weights of gravid uteri in the 2.0 and 2.5 mg/kg groups were significantly lower than the control value. Hematocrit values were not altered by lewisite treatment.

TABLE 9. Body Weights (g, Mean ± SE) of Pregnant Survivors in the Dose-Range Study of Lewisite in Rats

Day of Gestation	Dose Level (mg/kg)				
	0	0.5	1.0	2.0	2.5
0	238 ± 7.1	234 ± 6.1	234 ± 5.5	238 ± 7.8	235 ± 8.8
6	275 ± 7.6	271 ± 5.7	265 ± 4.2	271 ± 8.2	273 ± 12.7
7	276 ± 6.3	272 ± 5.9	261 ± 3.4	265 ± 7.2	267 ± 10.0
8	277 ± 6.7	277 ± 5.8	263 ± 3.1	263 ± 7.7	268 ± 9.7
9	280 ± 7.7	277 ± 6.2	266 ± 4.1	269 ± 8.4	270 ± 10.4
10	283 ± 7.4	285 ± 6.2	275 ± 3.1	273 ± 7.4	275 ± 10.5
11	289 ± 6.9	290 ± 6.5	275 ± 5.4	272 ± 8.2	279 ± 10.7
12	294 ± 6.9	292 ± 7.9	279 ± 5.6	269 ± 12.2	285 ± 10.5
13	299 ± 7.2	299 ± 8.5	285 ± 5.5	267 ± 16.5	289 ± 12.3
14[a]	303 ± 7.3	304 ± 8.3	288 ± 6.0	273 ± 17.5	286 ± 11.2
15[a]	311 ± 8.1	309 ± 9.4	300 ± 5.9	279 ± 18.3	287 ± 13.3
16[a]	321 ± 8.8[b]	317 ± 8.9[b]	307 ± 6.5[b]	288 ± 20.0[bc]	288 ± 14.1[c]
20[a]	382 ± 10.5[b]	375 ± 11.4[b]	371 ± 9.7[b]	339 ± 21.9[bc]	314 ± 32.8[c]

[a] Significant linear trend of decreased body weights with increasing dose levels.

[b-c] Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

TABLE 10. Maternal Measures (Mean ± SE) for Rats in the Dose-Range Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.5	1.0	2.0	2.5
Number of pregnant survivors	6	9	8	6	5
Body weight (g)					
0 dg	238 ± 7.1[a]	234 ± 6.1[a]	234 ± 5.5[a]	238 ± 7.8[ab]	235 ± 8.8[b]
20 dg	382 ± 10.5[a]	375 ± 11.4[a]	371 ± 9.7[a]	339 ± 21.9[ab]	314 ± 32.8[b]
Extragestational[c]	308 ± 9.5[a]	310 ± 8.7[a]	296 ± 6.2[ab]	295 ± 13.7[ab]	272 ± 18.5[b]
Extragestational[c] gain	70 ± 7.9	76 ± 4.6[a]	62 ± 6.6[ab]	56 ± 10.7[ab]	37 ± 23.2[b]
Weight of gravid uterus (g)	74 ± 6.8[a]	65 ± 6.7[ab]	74 ± 5.6[a]	45 ± 12.1[b]	42 ± 16.4[b]
Hematocrit (%)	40 ± 1.6	39 ± 0.8	38 ± 1.1	39 ± 0.8	40 ± 2.77

[ab]Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).
[c] Extragestational body weight = weight at 20 dg - weight of gravid uterus; extragestational gain = extragestational weight - weight at 0 dg.

The number of corpora lutea (CL) was highest in dams of the 1.0 mg/kg dose group (Table 11) and was significantly higher than the value for rats that received 2.5 mg/kg. Since the animals were not exposed to lewisite until 6 dg, this finding was not considered to be treatment related, but does explain the significantly lower values for implantation sites/dam observed in the 2.5 mg/kg group (Table 11). Preimplantation loss (based on percentage of implants/CL) tended to increase, but was not significantly increased, in the higher dose groups.

Intrauterine mortality tended to be higher in the rats exposed with 2.0 and 2.5 mg of lewisite/kg (Table 11). In the 2.0 mg/kg group, the percentage of resorptions during mid-gestation was significantly elevated; resorptions in the 2.5 mg/kg group tended to increase in the early stage of gestation. The increase in intrauterine mortality in these higher dose groups led to a significant reduction in the number of live fetuses per litter (Table 11); however, the smaller number of implantation sites/dam in the 2.5 mg/kg rats undoubtedly contributed to the reduced litter size. Reproductive measures for individual animals are presented in Table B1 of Appendix B.

TABLE 11. Reproductive Measures (Mean ± SE) for Rats in the Dose Range Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.5	1.0	2.0	2.5
Number of pregnant survivors	6	9	8	6	5
Corpora lutea/dam	18 ± 0.6[ab]	16 ± 1.0[ab]	19 ± 0.8[a]	18 ± 2.0[ab]	14 ± 2.0[b]
Implantations/dam	15 ± 1.2[a]	14 ± 1.0[ab]	16 ± 1.1[a]	13 ± 1.7[ab]	10 ± 3.1[b]
Implantations/CL (%)	86 ± 6.8	91 ± 2.7	81 ± 5.4	74 ± 8.9	66 ± 18.3
Resorptions (%)					
Early	6.5 ± 3.0[a]	13.6 ± 7.6[a]	4.3 ± 1.9[a]	5.5 ± 3.4[b]	24.0 ± 19.2[a]
Mid	0	0	0.7 ± 0.6	28.0 ± 16.4	1.3 ± 1.3
Late	0	0	0	1.1 ± 1.1	0
Total	6.5 ± 3.0	13.6 ± 7.6	5.0 ± 2.1	34.6 ± 16.2	25.3 ± 19.0
Litters with Resorptions (%)	67	56	50	67	60
Live fetuses/litter	15 ± 1.5[a]	12 ± 1.4[ab]	15 ± 0.9[a]	8 ± 2.4[b]	8 ± 3.1[b]
Live fetuses/implantation (%)	93.5 ± 3.0	86.5 ± 7.6	95.1 ± 2.1	65.5 ± 16.2	74.7 ± 19.0
Dead fetuses/litter	0	0	0	0	0

[a-b]Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

In litters exposed to the highest dose of lewisite, values for fetal body weights (Table 12) tended to be the lowest, and were significantly lower than values for fetuses of the 0.5 mg/kg group. Fetuses of the rats used in this study were remarkably free from gross anomalies (Table 12). Gross examinations of all fetuses revealed no abnormalities other than a vestigial tail in one fetus of the vehicle control group. Several stunted pups were observed: one (1.68 g) in the 1.0 mg/kg group; three (1.74-2.11 g) in one litter of the 2.0 mg/kg group; and two (1.00 and 1.04 g) in one litter of the

TABLE 12. Fetal Measures (Mean ± SE) for Rats in the Dose-Range Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.5	1.0	2.0	2.5
Number of litters examined	6	9	8	6	5
Number of fetuses examined	87	110	118	49	42
Body weight (g)					
Female	3.22^{ab} ± 0.12	3.35^{a} ± 0.08	3.22^{ab} ± 0.10	2.98^{ab} ± 0.38	2.62^{b} ± 0.58
Male	3.29^{ab} ± 0.08	3.60^{a} ± 0.06	3.37^{ab} ± 0.12	3.47^{ab} ± 0.12	2.86^{b} ± 0.65
Both sexes	3.24^{ab} ± 0.09	3.48^{a} ± 0.07	3.29^{ab} ± 0.10	3.13^{ab} ± 0.33	2.74^{b} ± 0.62
Sex ratio (% males)	45.5 ± 4.1	49.2 ± 5.9	48.5 ± 6.4	46.2 ± 16.1	40.2 ± 10.4
Gross anomalies[c]					
Vestigial tail	1/1	---	---	---	---
Stunted	---	---	1/1	3/1	2/1

[a-b] Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

[c] Number of anomalies over total number of litters with anomalies.

2.5 mg/kg group. It was noted that dams of these litters were dyspneic at some interval during gestation and that a lung lesion was found in rat 296 of the 2.5 mg/kg group at necropsy. Comparable fetal weight values ranged from 1.45 to 2.12 g for one control animal (#331), that was found to have a perforated lung at necropsy.

Since there appeared to be evidence of the induction of significant maternal and fetal mortality and toxicity at the higher dose levels of 2.0

and 2.5 mg/kg, dose levels of 0.5, 1.0 and 1.5 mg/kg were selected for the teratology study.

TERATOLOGY STUDY IN RATS

No deaths attributable to lewisite toxicity occurred during this study. Dosing trauma was the probable cause of death in 3% of the maternal animals (one control rat and two rats in the 1.0 mg/kg dose group) and an overall pregnancy rate of 76% was achieved (Table 13). Abnormalities were observed in two survivors; a mottled liver was evident in one rat of the 1.0 mg/kg dose group and bilateral hydronephrosis was observed in an animal dosed with 1.5 mg/kg (Table B2 of Appendix B).

TABLE 13. Status of Rats in the Teratology Study of Lewisite

Observed	Dose Level (mg/kg)			
	0	0.5	1.0	1.5
Number of rats dosed	25	25	25	25
Number of rats:				
Not pregnant	6	5	8	7
Pregnant (%)	19(76)	20(80)	17(68)	18(72)
Number of Survivors				
Pregnant	19	20	15	18
Not pregnant	5	5	8	7
Total	24	25	23	25

No significant differences among treatments in body weights of maternal animals were evident throughout the experimental period (Table 14). Results from other measures to detect signs of maternal toxicity (Table 15) revealed that lewisite exposure did not alter extragestational weights, weights of gravid uteri and hematocrit values, and that extragestational gains in rats that received the highest dose level of lewisite were significantly greater than those of the control group. This latter finding may have resulted from the removal of body weight values for nonpregnant animals from the original, randomly-assigned treatment means.

Reproductive measures, including numbers of corpora lutea and implantation sites, the percentage of resorptions, numbers of live and dead fetuses per litter and the percentage of live fetuses were not affected by lewisite treatment (Table 16). No differences among treatments were observed for fetal body weights, crown-rump lengths, fetal sex ratios or placental weights (Table 17).

TABLE 14. Body Weights (g, Mean ± SE) of Pregnant Survivors in the Teratology Study of Lewisite in Rats[a]

Day of Gestation	Dose Level (mg/kg) 0	0.5	1.0	1.5
	N = 19	N = 20	N = 15	N = 18
0	235 ± 3.3	236 ± 2.9	234 ± 2.9	235 ± 2.8
6	276 ± 4.0	280 ± 3.9	275 ± 3.9	278 ± 3.5
7	277 ± 4.1	282 ± 3.8	270 ± 4.7	277 ± 3.7
8	280 ± 4.6	284 ± 3.9	274 ± 4.6	279 ± 4.4
9	284 ± 4.2	290 ± 3.9	276 ± 5.2	285 ± 4.4
10	289 ± 5.0	294 ± 3.8	282 ± 5.1	292 ± 4.2
11	293 ± 5.8	302 ± 3.8	289 ± 6.0	300 ± 4.5
12	298 ± 5.5	309 ± 3.7	295 ± 6.3	306 ± 4.8
13	304 ± 5.2	312 ± 4.2	303 ± 6.6	312 ± 4.9
14	311 ± 5.2	318 ± 4.1	308 ± 6.3	318 ± 4.8
15	317 ± 5.4	325 ± 4.7	316 ± 6.3	326 ± 4.9
16	325 ± 6.0	334 ± 5.3	325 ± 6.5	337 ± 5.3
20	373 ± 8.7	390 ± 8.6	378 ± 8.7	397 ± 7.8

[a]No significant differences ($P \geq 0.05$) were found among treatment groups (Duncan's multiple range test).

TABLE 15. Maternal Measures (Mean ± SE) for Rats of the Teratology Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.5	1.0	1.5
Number of pregnant survivors	19	20	15	18
Body weight (g)				
0 dg	234 ± 3.2	236 ± 2.9	232 ± 3.0	235 ± 2.8
20 dg	373 ± 8.7	390 ± 8.6	378 ± 8.7	397 ± 7.8
Extragestational[a]	301 ± 4.5	312 ± 4.6	304 ± 5.5	314 ± 5.0
Extragestational gain[a]	65.8 ± 3.0[b]	75.2 ± 4.3[bc]	69.4 ± 4.7[bc]	79.2 ± 4.3[c]
Weight of gravid uterus (g)	72.2 ± 6.4	78.9 ± 6.9	74.7 ± 7.7	82.8 ± 5.2
Hematocrit (%)	36.3 ± 0.8	35.6 ± 0.6	36.5 ± 1.0	36.0 ± 0.8

[a] Extragestational body weight = weight at 20 dg - weight of gravid uterus; extragestational gain = extragestational weight - weight at 0 dg.
[bc]Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

TABLE 16. Reproductive Measures (Mean ± SE) for Rats of the Teratology Study of Lewisite[a]

Observation	Dose Level (mg/kg) 0	0.5	1.0	1.5
Number of pregnant survivors	19	20	15	18
Corpora lutea/dam	16 ± 1.1	17 ± 0.6	16 ± 0.8	17 ± 0.9
Implantations/dam	14 ± 1.1	14 ± 1.2	14 ± 1.2	15 ± 1.0
Implantations/corpus luteum (%)	85 ± 4.9	82 ± 5.9	85 ± 6.2	89 ± 4.1
Resorptions (%)				
Early	7.6 ± 2.5	8.3 ± 3.1	8.1 ± 4.5	7.9 ± 2.9
Mid	1.7 ± 1.1	1.1 ± 0.5	1.0 ± 0.7	0.3 ± 0.3
Late	0	0	0	0
Total	9.2 ± 2.8	9.4 ± 3.0	9.1 ± 4.5	8.5 ± 2.9
Litters with Resorptions (%)	58	55	53	55
Live fetuses/litter	12 ± 1.1	13 ± 1.0	13 ± 1.3	14 ± 1.0
Live fetuses/implantation (%)	90.8 ± 2.8	90.6 ± 3.0	90.9 ± 4.5	91.5 ± 2.9
Dead fetuses/litter	0	0	0	0

[a]No significant differences ($P \geq 0.05$) were found among treatment groups (Duncan's multiple range test).

Exposure of maternal rats to lewisite at dose levels as high as 1.5 mg/kg did not induce significant numbers of morphologic alterations in their fetuses (Table 18). Major malformations were observed in two fetuses of the control group and two fetuses of the 1.0 mg/kg group. The number of fetuses in the 0.5 and 1.0 mg/kg dose groups with reduced sternebral ossification was larger than that of the control group but retarded pelvic ossification was more evident in control and low-dose fetuses than in fetuses exposed to the two higher doses of lewisite. In both instances of reduced ossification, significance based on the number of affected litters, which is considered to be more appropriate for the determination of statistical differences than fetal incidence, was not achieved.

TABLE 17. Fetal and Placental Measures (Mean ± SE) for the Teratology Study of Lewisite in Rats[a]

Observation	Dose Level (mg/kg) 0	0.5	1.0	1.5
Number of litters examined	19	20	15	18
Number of fetuses examined	235	267	195	258
Body weight (g)				
Female	3.74 ± 0.13	3.88 ± 0.12	3.65 ± 0.13	3.69 ± 0.09
Male	3.88 ± 0.13	4.08 ± 0.11	3.87 ± 0.15	3.94 ± 0.07
Both sexes	3.81 ± 0.13	3.97 ± 0.11	3.78 ± 0.14	3.82 ± 0.08
Sex ratio (% male)	46 ± 2.9	49 ± 3.8	48 ± 4.8	49 ± 3.6
Crown-rump length (mm)				
Female	35.9 ± 0.6	36.9 ± 0.5	35.9 ± 0.5	36.0 ± 0.4
Male	36.6 ± 0.5	37.6 ± 0.5	36.6 ± 0.5	36.9 ± 0.4
Both sexes	36.2 ± 0.5	37.1 ± 0.5	36.4 ± 0.6	36.3 ± 0.3
Placental weight (mg)	519 ± 30	534 ± 36	521 ± 32	499 ± 19

[a]No significant differences ($P \geq 0.05$) were found among treatment groups (Duncan's multiple range test).

TABLE 18. Incidence of Fetal Morphologic Alterations (Number of Fetuses/Litters) in the Teratology Study of Lewisite in Rats

Observation	Dose Level (mg/kg) 0	0.5	1.0	1.5
Number of examined				
Litters	19	20	15	18
Fetuses	235	267	195	258
Malformations				
Exencephaly	1/1	-	-	-
Edema	-	-	1/1[b]	-
Multiple	1/1[a]	-	1/1[b]	-
Total malformed	2/1	0	2/2	0
Minor Anomalies				
Anomalous ribs[c]	1/1	3/2	2/1	2/2
Misaligned sternebrae	1/1	1/1	-	-
Variations				
Hydroureter	4/3	7/5	6/3	8/5
Supernumerary ribs[d]	34/9	35/10	24/7	38/9
Reduced ossification				
Skull	1/1[a]	-	1/1	-
Sternebrae #1-4	8/6	28[e]/7	19[e]/9	13/5
Thoracic vertebrae	38/13	31/14	20/9	54/12
Pelvis	10/3	11/2	1[e]/1	2[e]/2
Phalanges	1/1	3/2	-	-

[a]Exencephaly, open eye, ocular dysgenesis, retrognathia and retroesophageal aortic arch observed in fetus 12 of litter 453.
[b]Exencephaly and spina bifida observed in fetus 4 of litter 563.
[c]Anomalous (wavy, bent or knobby) ribs.
[d]Include extra and rudimentary ribs and ossification sites, all at lumbar #1.
[e]Significantly different ($P \leq 0.05$) from control fetuses, using a chi-square test.

DOSE-RANGE STUDY IN RABBITS

The pregnancy rate for this study was 90%, but all of the animals that were not pregnant were in one dose group, the vehicle control (Table 19). Mortality associated with lewisite toxicity (Figure 1) was 0, 86, 100 and 100% for treatment groups that received 0.5, 1.0, 1.5 and 2.0 mg/kg, respectively. Median times of death where 15, 11 and 9 dg for the three highest dose groups.

Typical lesions resulting from lewisite exposure included inflammation and hemorrhage of the mucosa in the pyloric and cardiac regions of the stomach (Table B3 of Appendix B). Mucosal inflammation was observed following four doses of 0.5 mg/kg and severe gastric edema was evident in one animal after 3 days of dosing with 2.0 mg/kg. In addition to the gastric lesions, duodenal hemorrhage was observed at necropsy on 13 dg and duodenal necrosis and hemorrhagic foci in the cecum were evident on 22 dg in animals of the 1.0 mg/kg dose group. Although no mortality was attributed to lewisite toxicity in the lowest dose group, hemorrhage of the gastric mucosa was observed at necropsy in three of the five animals that died from dosing trauma on 10, 14 and 16 dg; inflammation of the gastric mucosa and excessive amounts of peritoneal or pericardial fluid were evident in two of three rabbits at scheduled sacrifice on 30 dg in this dose group.

TABLE 19. Status of Rabbits in the Dose-Range Study of Lewisite

Observation	Dose Level (mg/kg)				
	0	0.5	1.0	1.5	2.0
Number of rabbits:					
Dosed	8	8	8	8	8
Pregnant (%)	4 (50)	8 (100)	8 (100)	8 (100)	8 (100)
Not pregnant	4	0	0	0	0
Number of survivors:					
Pregnant	3[a]	3	1	0	0
Not pregnant	4	0	0	0	0
Number of deaths:					
Pregnant	1	5	7	8	8
Not pregnant	0	0	0	0	0
Probable cause of death:					
Dosing trauma[b]	1	5	1	3	0
Toxicity	-	0	6	5	8

[a]Lung dose in one survivor (597) on 14 dg; all data omitted after this time.
[b]Dose delivered into the lungs or perforation of the lung or trachea.

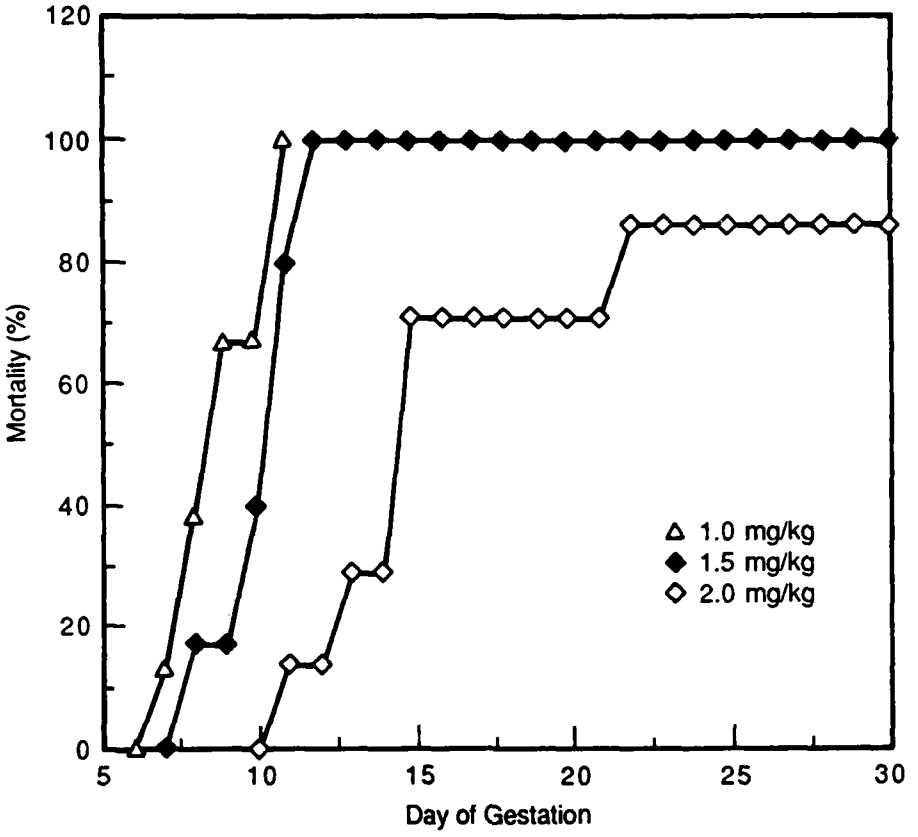

Figure 1. Cumulative Mortality Attributed to Lewisite Toxicity in Rabbits of the Dose-Range Study (Percent mortality = 100 x number of deaths from toxicity/total number of rabbits - number of deaths from other causes on successive days).

Significant decreases in maternal body weight gains of survivors and non-survivors were evident in all lewisite-dosed animals after two days of treatment (Figure 2). Limited sample sizes, resulting from death of the animals, did not permit statistical comparisons after 14 dg.

Maternal, reproductive and fetal parameters in the limited number of surviving does with live litters are shown in Tables 20 and 21. Maternal body weights of the lewisite dosed animals were minimal during the last part of the dosing period (15 to 20 dg). By 30 dg body weights had recovered to their original value at 0 dg, but calculations of extragestational gains demonstrated that body weight losses were sustained by all treatment groups and were largest in the animal dosed with 1.0 mg/kg.

Qualitative comparisons for reproductive performance the of small number of control and treated survivors (Table 21 and Table C3 of Appendix C) did

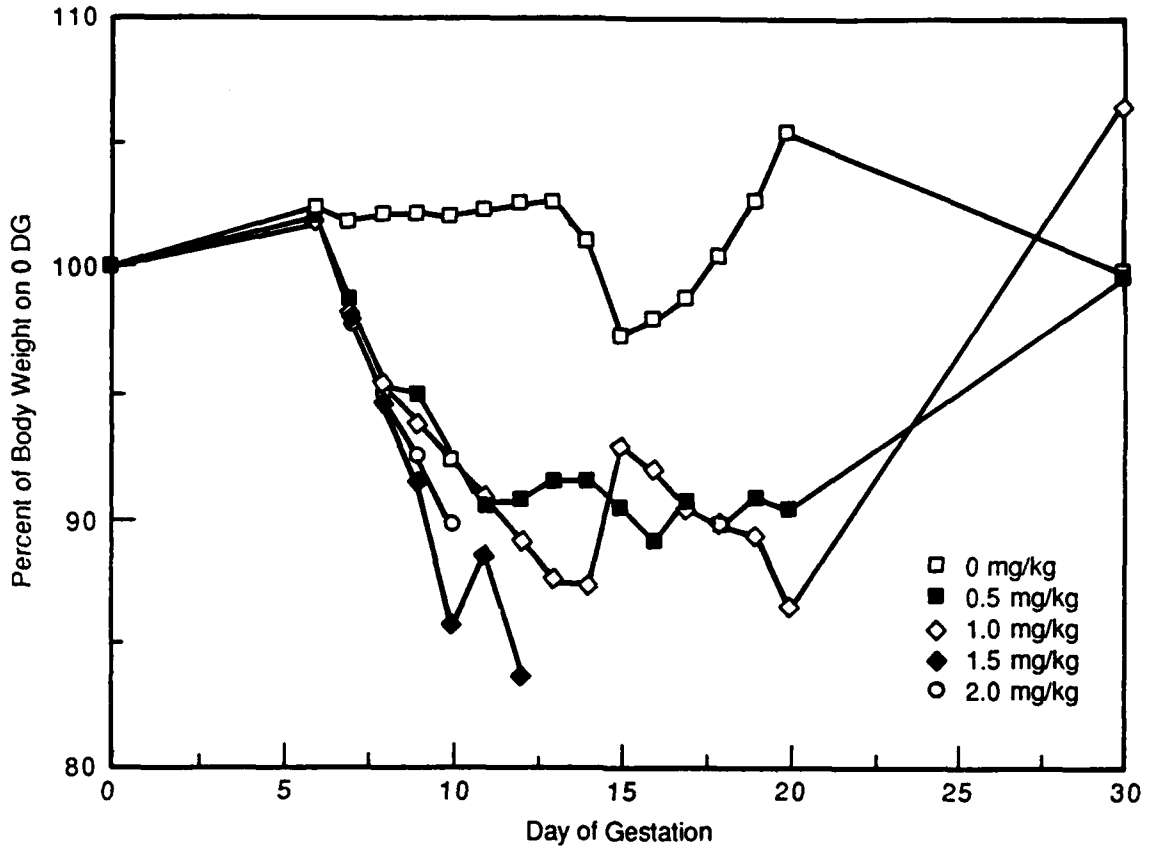

Figure 2. Weight Gain of Maternal Rabbits (Survivors and Non-Survivors) in the Dose-Range Study of Lewisite (Values for all lewisite dosed animals were significantly lower than control values from 8 to 14 dg. There were too few survivors for statistical evaluation from 15 to 30 dg).

not suggest that the lewisite treatments affected intrauterine mortality, fetal viability or fetal body weights. External examinations of fetuses revealed no gross anomalies, even in the litter that survived exposure to 1.0 mg/kg.

Selection of the highest dose for the teratology study was based on results from the 0.5 mg/kg rabbits which included an overt depression in maternal body weight and no mortality attributable to lewisite toxicity (although a number of lesions, such as inflammation and hemorrhage of the gastric mucosa, and the accumulation of excessive amounts of fluid, were observed at necropsy). Furthermore, embryo mortality did not appear to be increased at this dose level so that the number of live fetuses per litter would be sufficient for the evaluations.

Table 20. Body Weights (kg, Mean ± SE) of Pregnant Survivors in the Dose-Range Study of Lewisite in Rabbits[a]

Day of Gestation	Dose Level (mg/kg)		
	0	0.5	1.0
	N = 2	N = 3	N = 1
0	4.09 ± 0.07	4.52 ± 0.23	4.20
6	4.15 ± 0.15	4.54 ± 0.26	4.30
7	4.10 ± 0.08	4.38 ± 0.20	4.18
8	4.11 ± 0.10	4.24 ± 0.19	4.11
9	4.11 ± 0.08	4.25 ± 0.20	4.17
10	4.13 ± 0.06	4.20 ± 0.24	4.16
11	4.15 ± 0.07	4.20 ± 0.27	3.99
12	4.14 ± 0.05	4.22 ± 0.27	3.92
13	4.14 ± 0.03	4.27 ± 0.25	3.86
14	4.03 ± 0.04	4.17 ± 0.28	3.86
15	3.98 ± 0.05	4.19 ± 0.30	3.82
16	4.00 ± 0.05	4.14 ± 0.28	3.77
17	4.04 ± 0.06	4.10 ± 0.27	3.87
18	4.10 ± 0.02	4.06 ± 0.25	3.94
19	4.19 ± 0.04	4.11 ± 0.25	3.95
20	4.31 ± 0.08	4.09 ± 0.19	3.92
30	4.12 ± 0.22	4.52 ± 0.36	4.29

[a]There were no survivors at dose levels of 1.5 and 2.0 mg/kg.

TABLE 21. Maternal, Reproductive and Fetal Measures (Mean ± SE) for the Dose-Range Study of Lewisite in Rabbits

Observation	Dose Level (mg/kg)		
	0	0.5	1.0
Number of pregnant survivors	2	3	1
Maternal weights (kg):			
Body weight at 0 dg	4.09 ± 0.07	4.52 ± 0.23	4.20
Body weight at 20 dg	4.12 ± 0.22	4.52 ± 0.36	4.29
Weight of gravid uterus	0.52 ± 0.09	0.67 ± 0.08	0.86
Extragestational weight	3.61 ± 0.13	3.85 ± 0.33	3.43
Extragestation gain	-0.48 ± 0.06	-0.67 ± 0.13	-0.77
Hematocrit (%)	41.5 ± 1.5	37.7 ± 3.2	42.0
Number/dam			
Corpora lutea	10.0 ± 1.0	12.7 ± 2.3	12.0
Implantation sites	9.5 ± 1.5	12.3 ± 2.0	12.0
Resorptions (%)			
Early	0	0	0
Late	6.3 ± 6.3	7.9 ± 4.0	0
Total	6.3 ± 6.3	7.9 ± 4.0	0
Number/litter			
Dead fetuses	0	0.33 ± 0.3	0
Live fetuses	9.0 ± 2.0	11.0 ± 1.5	12.0
Fetal body weights (g)	41.5 ± 1.2	44.0 ± 5.0	54.9 ± 5.3[a]

[a]Within litter variability

Since all of the dose levels used in this dose-range study induced significant maternal effects, the selection of a dose for a "no effect level" for the teratology study was made by comparing data from the rat and rabbit studies. This evidence indicated that the maternal response to lewisite exposure could be correlated with the severity of the gastric lesions and, therefore, the concentration of the dosing solution rather than the dose/kg was apparently the most important consideration. A concentration of 0.45 mg/ml (dose level of 1.5 mg/kg) in the rat and 0.11 mg/kg in the rabbit did not induce maternal or fetal effects. Subsequently, a wider range of dose levels, 0.07, 0.02, and 0.6 mg/kg (solution concentrations of 0.28, 0.8 and 2.4 mg/ml), was selected for the rabbit teratology study.

TERATOLOGY STUDY IN RABBITS

The pregnancy rate for this study was 69% (Table 22). No corpora lutea were observed at necropsy in 23% of the rabbits and, implantation failure was evident in 11% of the animals with corpora lutea (Table C4 of Appendix C).

TABLE 22. Status of Rabbits in the Teratology Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.07	0.2	0.6
Number of Rabbits:				
Dosed	19	18	18	19
Pregnant (%)	12 (63)	11 (61)	13 (72)	15 (79)
Not pregnant	7	7	5	4
Number of survivors:	18	11	7	5
Pregnant	9	6	5	3
Aborting[a]	2	1	0	0
Not pregnant	7	4	2	2
Number of deaths:	1	7	11	14
Pregnant	1	4	8	12
Not pregnant	0	3	3	2
Probable cause of death[b]:				
Lewisite toxicity	0	2	6	11
Dosing trauma	0	4	3	3
Other[c]	1	1	2	0

[a]Number of abortions is not included in the number of survivors.
[b]Observations at necropsy are listed in Table B4 of Appendix B.
[c]Includes stress, handling trauma and complications of pregnancy.

Dosing trauma (delivery of the dose to the lung or damage to tissues of the respiratory tract during dosing) accounted for 14% of the deaths that occurred during the study. Other causes of death in 5% of the range of dose levels, rabbits were stress or handling trauma, and complications of pregnancy, such as infection and abortion (Table 22 and Table B4 of Appendix B).

On the basis of the results from the dose range study, the number of deaths which were attributed to lewisite toxicity was much higher than expected. The mortality rate (corrected for deaths from other causes) was 13, 46 and 69% in rabbits dosed with 0.07, 0.2 and 0.6 mg/kg, respectively, (Figure 3). Median times of death for these treatment groups were 18, 20 and 16 dg, respectively. Gastric inflammation, hemorrhage, edema and necrosis were observed at necropsy; gastric ulceration and inflammation of the mucosa and blood vessels of the duodenum and cecum were observed less frequently.

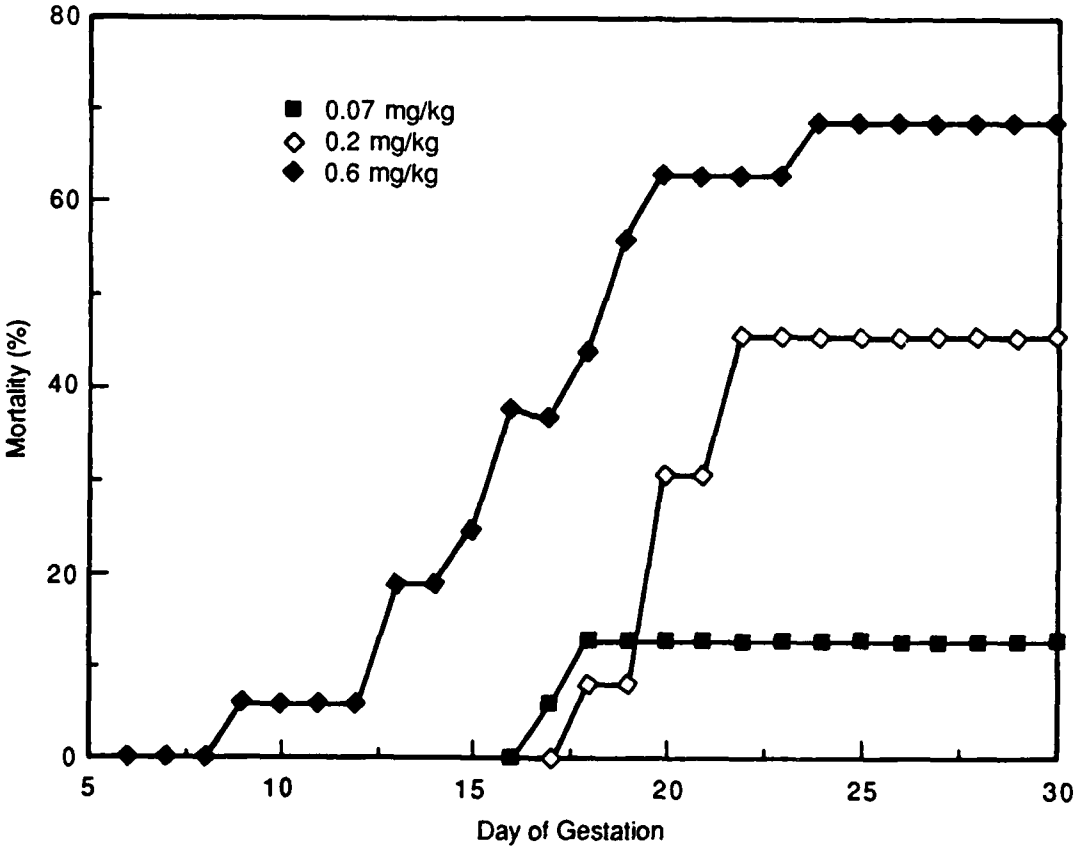

Figure 3. Cumulative Mortality Attributed to Lewisite Toxicity in Rabbits of the Teratology Study. (Percent mortality = 100 x number of deaths from toxicity/total number of rabbits - number of deaths from other causes on successive days).

Maternal body weight gains in all pregnant animals (survivors and non-survivors) of the animals dosed with 0.07 and 0.2 mg/kg did not differ from control values throughout the experimental period, but weight gains for rabbits in the highest dose group were significantly lower than control values from 11 through 20 dg (Figure 4). When absolute values for body weights, extragestational weights and extragestational gains of pregnant survivors (Table 23 and 24) were analyzed, no significant differences among treatments were observed, but when weight gains (based on the percentage of the body weight at 0 dg) were considered, values for animals in the 0.6 mg/kg group were significantly less than those of all other treatments from 12 through 20 dg (Figure 5). Qualitative observations for anorexia (Table 25) showed that a large percentage of the rabbits in the highest dose group were anorectic during dosing and that anorexia tended to be observed more frequently in all lewisite-treated animals from 20 to 30 dg.

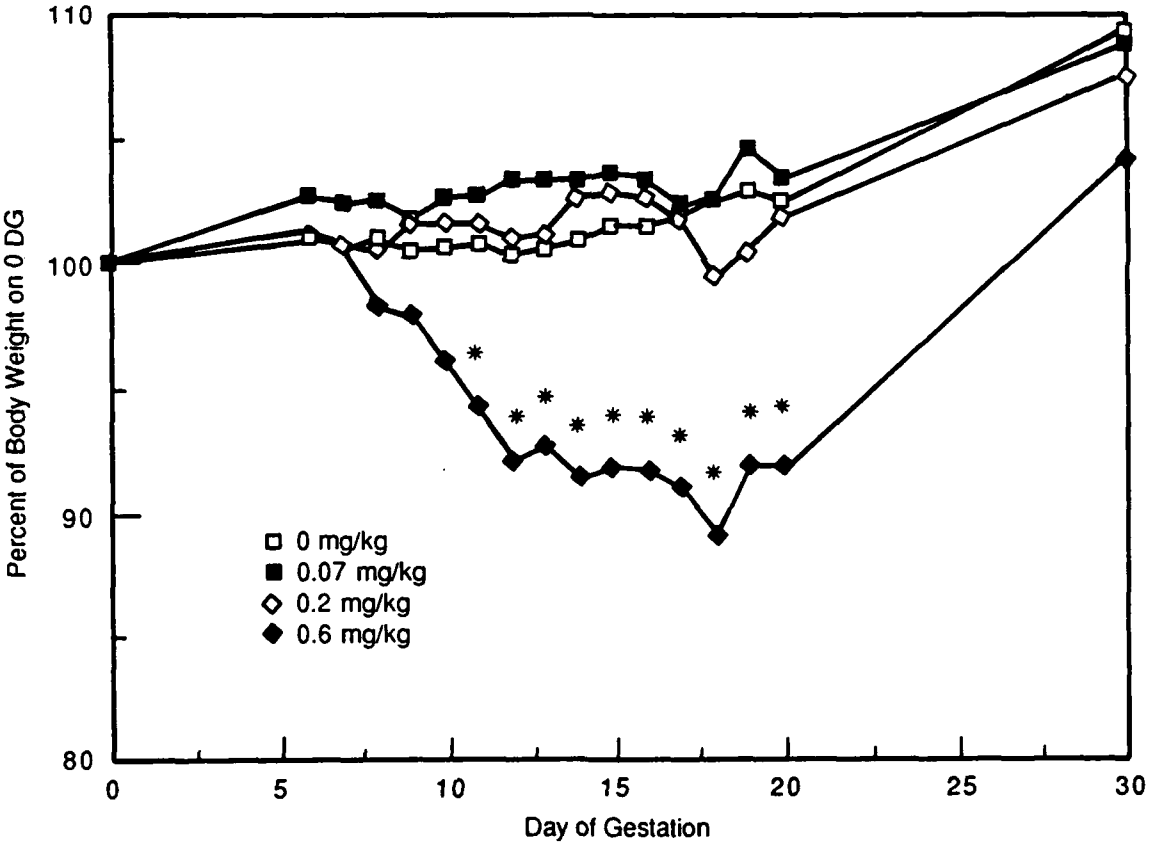

Figure 4. Weight Gain of Maternal Rabbits (Survivors and non-survivors) in the Teratology Study of Lewisite. *Significantly different from control value ($P < 0.05$, Randomized Test).

TABLE 23. Body Weights (kg, Mean ± SE) of Pregnant Survivors in the Teratology Study of Lewisite in Rabbits[a]

Day of Gestation	Dose Level (mg/kg) 0	0.07	0.2	0.6
	N = 9	N = 6	N = 5	N = 3
Pre-	3.65 ± 0.08	3.59 ± 0.05	3.59 ± 0.10	3.77 ± 0.25
0	3.92 ± 0.08	3.85 ± 0.04	3.96 ± 0.17	4.03 ± 0.26
6	3.93 ± 0.09	4.00 ± 0.05	3.96 ± 0.14	4.10 ± 0.22
7	3.93 ± 0.10	3.98 ± 0.05	3.94 ± 0.17	4.10 ± 0.22
8	3.94 ± 0.09	4.00 ± 0.05	3.90 ± 0.17	3.96 ± 0.26
9	3.94 ± 0.10	4.01 ± 0.06	3.94 ± 0.18	3.94 ± 0.29
10	3.95 ± 0.10	4.02 ± 0.07	3.92 ± 0.16	3.89 ± 0.32
11	3.94 ± 0.11	4.03 ± 0.06	3.95 ± 0.16	3.86 ± 0.35
12	3.95 ± 0.11	4.04 ± 0.05	3.92 ± 0.16	3.76 ± 0.35
13	3.96 ± 0.12	4.04 ± 0.06	3.97 ± 0.19	3.79 ± 0.34
14	3.96 ± 0.11	4.01 ± 0.09	3.99 ± 0.18	3.73 ± 0.36
15	3.98 ± 0.12	4.01 ± 0.09	4.00 ± 0.17	3.80 ± 0.35
16	4.00 ± 0.12	4.01 ± 0.09	4.03 ± 0.18	3.73 ± 0.39
17	4.02 ± 0.12	3.99 ± 0.09	4.03 ± 0.17	3.74 ± 0.39
18	4.06 ± 0.12	3.98 ± 0.08	4.01 ± 0.17	3.79 ± 0.36
19	4.08 ± 0.12	4.06 ± 0.10	4.02 ± 0.16	3.72 ± 0.38
20	4.08 ± 0.13	4.04 ± 0.10	4.04 ± 0.19	3.71 ± 0.26
30	4.28 ± 0.12	4.18 ± 0.08	4.26 ± 0.19	4.19 ± 0.23

[a]No significant differences ($P \geq 0.05$) were found among treatment groups (Duncan's multiple range test).

TABLE 24. Maternal Measures (Mean ± SE) for Rabbits in the Teratology Study of Lewisite

Observation	Dose Level (mg/kg) 0	0.07	0.2	0.6
Number of pregnant survivors	9	6	5	3
Body weight (kg)				
0 dg[a]	3.92 ± 0.08	3.85 ± 0.04	3.96 ± 0.17	4.03 ± 0.26
30 dg	4.28 ± 0.12	4.18 ± 0.08	4.26 ± 0.19	4.19 ± 0.23
Extragestational[b]	3.86 ± 0.13	3.88 ± 0.10	3.84 ± 0.14	3.89 ± 0.14
Extragestational gain[c]	-0.05 ± 0.08	0.03 ± 0.13	-0.12 ± 0.15	-0.14 ± 0.14
Weight of gravid uterus (kg)	0.42 ± 0.06	0.30 ± 0.09	0.42 ± 0.12	0.30 ± 0.10
Hematocrit (%)[d]	43 ± 0.9	42 ± 3.0	37 ± 1.7	33 ± 0

[a]Days of gestations (dg)
[b]Extragestational body weight = body weight at 30 dg - weight of gravid uterus
[c]Extragestation gain = extragestational body weight - body weight at 0 dg
[d]Significant ($P \leq 0.05$) trend of decreasing values with increasing dose levels (orthogonal contrast test); samples for one sacrifice day were lost (N = 6, 5, 3, and 3 for 0, 0.07, 0.2 and 0.6 mg/kg groups respectively).

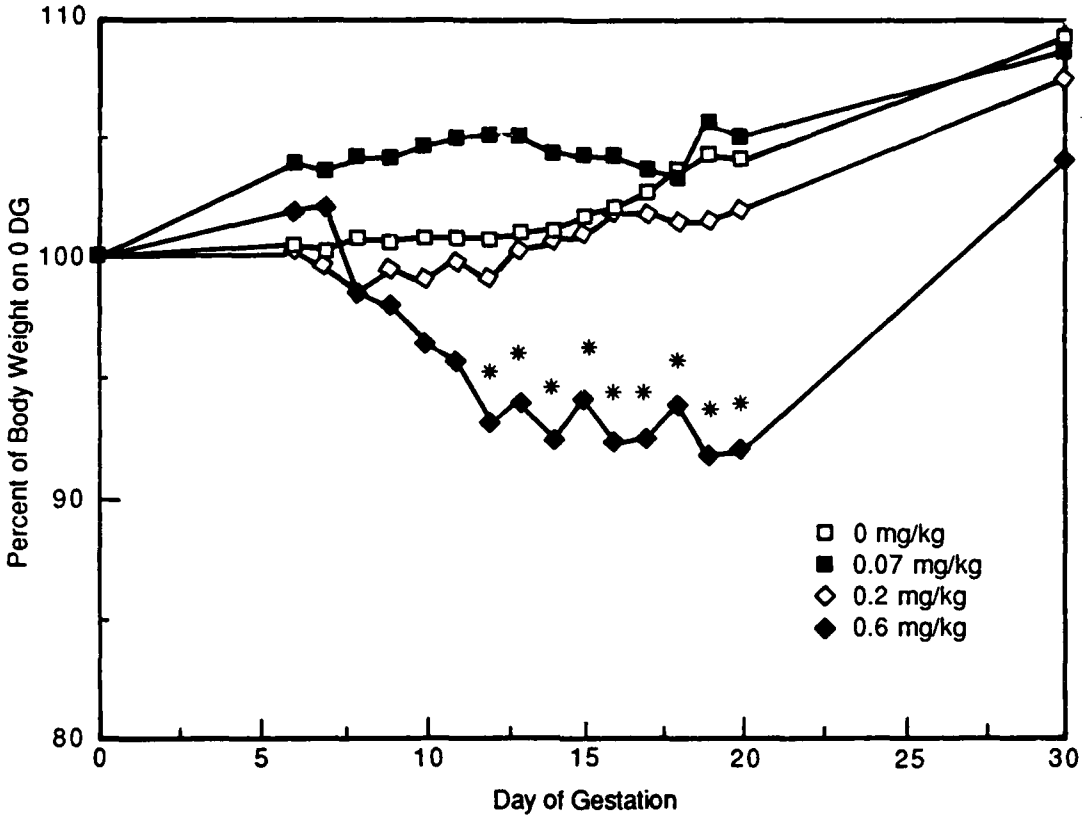

Figure 5. Weight Gain of Surviving Maternal Rabbits in the Teratology Study of Lewisite. *Significantly different from control value ($P < 0.05$).

TABLE 25. Anorexia[a] in Pregnant Rabbits of the Teratology Study of Lewisite

Days of Gestation	Dose Level (mg/kg)			
	0	0.07	0.2	0.6
6 - 12	$4.8 \pm 3.6^{b}_{b}$	1.3 ± 1.3^{b}	$2.4 \pm 1.6^{b}_{b}$	41.4 ± 6.7^{c}
13 - 19	$9.0 \pm 2.8^{b}_{b}$	13.7 ± 4.4^{b}	$12.1 \pm 5.5^{b}_{b}$	$53.3 \pm 4.6^{c}_{c}$
20 - 30	5.4 ± 2.0^{b}	25.8 ± 6.6^{c}	19.9 ± 5.4^{bc}	23.3 ± 7.9^{bc}

[a] Percentage of animal-days of anorexia, mean ± SE.

[bc] Values that do not share a common letter superscript are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

A significant trend of decreasing hematocrit values with increasing dose levels of lewisite was evident in the maternal animals (Table 24). Results from observations for reproductive measures (Table 26) showed that numbers of corpora lutea and implantation sites did not differ among treatments, but the percentage of implantations/corpus luteum in the 0.6 mg/kg animals was lower than values for other lewisite-treated rabbits. This finding was not believed to be treatment-related, but probably was a result of the small number of pregnant survivors. Intrauterine mortality tended to be higher in animals dosed with lewisite, but no significant differences were observed because samples sizes were small and within-sample variabilities were large.

Determinations of significant differences in fetal measures among treatment groups were also limited by the small number of surviving litters. Although not significantly different, placental weights and fetal body weights and crown-rump lengths tended to be lower in the higher dose groups. Sex ratio appears to be lower in lewisite-dosed fetuses, but significance was not achieved (Table 27). The incidence of major malformations and anomalies was very low in these rabbits, even in fetuses of the high dose group (Table 28). Although absolute values for body weights were not significantly different, the incidence of stunted fetuses (body weight $\leq$2 SD of the mean body weight) was significantly increased for fetuses and litters of the 0.6 mg/kg animals (Table 28). In this treatment group, incidences of fetuses with supernumerary ribs and reduced pelvic ossification were significantly higher.

TABLE 26. Reproductive Measure (Mean ± SE) for Rabbits in the Teratology Study of Lewisite

Observations	Dose Level (mg/kg) 0	0.07	0.2	0.6
Number of rabbits				
Without corpora lutea	7	4[a]	4	2
With corpora lutea/no implantation sites	0	3	1	2
Pregnant (%)	12[b] (63)	11[b] (61)	13 (72)	15 (79)
Pregnant survivors	9[b]	6[b]	5	3
Number of				
Corpora lutea/doe	10.7 ± 1.4	7.5 ± 2.6	8.4 ± 2.1[c]	11.7 ± 2.4
Implantation/doe	8.0 ± 1.4	6.8 ± 2.0	8.6 ± 2.3[c]	7.0 ± 2.1
Implantation/corpus luteum (%)	74.4 ± 6.7[ef]	84.9 ± 7.4[de]	100.8 ± 5.0[d]	57.0 ± 7.2[f]
Resorptions (%)				
Early	6.7 ± 4.9	18.2 ± 16.4	20.0 ± 20.0	11.1 ± 11.1
Late	4.3 ± 2.2	14.8 ± 10.6	3.1 ± 3.1	23.6 ± 11.9
Total	11.0 ± 5.9	33.0 ± 16.6	23.1 ± 19.5	34.7 ± 19.3
Litters with resorptions (%)	56	67	40	67
Number/litter				
Dead fetuses	0	0.7 ± 0.7	0	0
Live fetuses	7.0 ± 1.3	3.8 ± 1.9	7.8 ± 2.4	5.3 ± 2.6
Live fetuses (%)	89.0 ± 5.9	61.0 ± 19.6	76.9 ± 19.5	65.0 ± 19.3

[a] All corpora lutea had regressed in doe 5903.
[b] Data were omitted for rabbits that aborted prior to sacrifice: 1739 and 3761 in the 0 mg/kg group and 3176 in the 0.07 mg/kg group.
[c] Numbers of implantation sites exceeded numbers of corpora lutea in rabbits 3202 and 5940.
[d-f] Values that do not share a common superscript letter are significantly different ($P \leq 0.05$) from one another (Duncan's multiple range test).

Table 27. Fetal Measures (Mean ± SE) for Rabbits in the Teratology Study of Lewisite[a]

Observation	Dose Level (mg/kg) 0	0.07	0.2	0.6
Number of				
Litters examined	9	4	4	3
Fetuses examined	63	23	39	16
Body weight (g)				
Females	44.1 ± 3.0	45.9 ± 5.2	41.1 ± 2.0	38.6 ± 9.3
Males	44.2 ± 2.4	46.8 ± 8.2	41.4 ± 2.6	38.7 ± 7.9
Crown-rump length (mm)				
Females	98 ± 3.4	96 ± 2.9	93 ± 3.0	89 ± 7.0
Males	98 ± 3.0	97 ± 4.3	94 ± 2.1	91 ± 3.7
Sex ratio (% Males)	52 ± 5.7	31 ± 16.3	42 ± 11.1	33 ± 17.6
Placental weight (g)				
Females	5.30 ± 0.46	5.74 ± 1.84	4.58 ± 0.56	4.97 ± 0.97
Males	5.54 ± 0.47	5.12 ± 1.39	4.32 ± 0.41	4.80 ± 0.70

[a]No significant differences ($P \geq 0.05$) were found among treatment groups (Duncan's multiple range test).

TABLE 28. Incidence of Morphologic Alterations in Rabbit Fetuses Exposed to Lewisite

Observation	Dose Levels (mg/kg) 0	0.07	0.2	0.6
Number of fetuses/ litters examined	63/9	23/4	39/4	16/3
Malformations				
Retrognathia/ macroglossia	1/1	--	--	--
Cardiac/major vessels defects (abdominal edema)	--	--	1/1[a]	--
Minor Anomalies				
Cerebral fold	1/1	--	--	--
Brachiocephalic artery missing	--	--	--	1/1
Vertebrae				
Agenesis/scoliosis	1/1[b]	--	--	--
supernumerary arch	1/1[b]	--	--	--
Sternebrae - misaligned fused	--	--	1/1[a]	--
Ribs - fused, branched	1/1	--	--	--
Forelimb flexure	--	1/1	1/1	1/1
Morphologic Variations				
Stunted Fetuses	--	--	2/2	5[c]/2[c]
Ribs				
Supernumerary	28/8[b]	6/2	11/2	13[c]/3
Ossification site at lumbar 1	3/1	2/1	--	--
Thickened distally	--	--	1/1[a]	--
Sternebrae				
Extra ossification site	--	--	1/1[a]	--
Reduced ossification	16/7	13/4	14/4	7/1
Pelvis - reduced ossification	--	--	--	3[c]/1

[a]Fetus 1 in litter 3202
[b]Fetus 3 in litter 3774
[c]Significantly different ($P \leq 0.05$) from control value using Fisher's Exact Test.

DISCUSSION

The pregnancy rate for the two lewisite studies in rats was 77% and was not appreciably different from a mean pregnancy rate of 80% for all four recent studies (sulfur mustard and lewisite) performed with CD rats in this facility. Despite the use of artificial insemination and the timed induction of ovulation with chorionic gonadotropin, pregnancy rates in rabbits were variable (64 to 90% in the four studies). The stock of rabbits, the source of chorionic gonadotropin and the techniques used for artificial insemination were the same for all studies. This variability in pregnancy rate appeared to be seasonal since the pregnancy rate was 90% in a study performed in the spring and ranged from 64 to 71% in studies performed in the fall and winter.

The dose levels of lewisite required to induce maternal mortality were remarkably different in rats and rabbits (Table 29). The lowest administered dose to cause death in rats was 2.0 mg/kg and was 0.07 mg/kg in rabbits. Probit analyses of maternal mortality data combined from dose-range and teratology studies of both species yielded LD_{50} values of 3.1 mg/kg for the rat and 0.26 mg/kg for the rabbit (Figure 6). The 95% lower Fiducial limit for rats was 2.5 and the lower and upper limits for rabbits was 0.16 and 0.40, respectively. However, in the case of the rat the 3.1 value is an extrapolation beyond the experimental data and an upper limit could not be estimated. Dose levels predicted to induce $\geq$95% mortality were 4.8 and 1.5 mg/kg in rats and rabbits, respectively. It should be noted that, below an LD_{50}, estimated dose levels to induce lethality were 11.9 to 13.3 times higher in rats than in rabbits and that the concentration of the solutions administered to the rabbits was 13.3 times greater than that of the solutions used in rat studies. This relationship was obviously not observed at higher dose levels, suggesting a threshold effect for a given experimental regimen.

Mortality data from the rat and rabbit studies also emphasized the value of conducting preliminary dose-range studies in a series of experimental blocks that can be adjusted to permit testing of a wider range of dose levels, particularly for agents that induce localized primary lesions. Since maternal mortality data were considered to be a good basis for the design of the studies and large differences in dose-response relationships were observed between rats and rabbits, the determination of a reliable mortality curve for each species was, in retrospect, of particular importance.

Assignment of a "probable cause of death" (PCOD) to individual animals, using only the gross observations at necropsy, was often difficult and, in some cases, may appear to be arbitrary (Appendix B). For rabbits, the use of a 22-in feeding tube, rather than a dosing needle, ensured that potential damage to the esophagus by lewisite did not occur and that multiple doses could be delivered to the stomach. However, this means of dose-delivery was one factor that led to higher than usual mortality rates due to dosing trauma (17% as compared to $\leq$8%). In considering statistics for lewisite lethality, the data from animals, whose immediate cause of death was dosing trauma, were not included because the eventual outcome of the experimental treatment could not be predicted. Except for one dose group, the 0.5 mg/kg rabbits in the

TABLE 29. Summary of Effects of Lewisite on Pregnant Rats and Rabbits

Dose Level (mg/kg)	Concentration (mg/ml)		Rat Studies		Rabbit Studies	
	Rat	Rabbit	Dose-Range	Teratology	Dose-Range	Teratology
0.07	-	0.28	-	-	-	13% mortality
0.2	-	0.8	-	-	-	46% mortality; hematocrit low[a]
0.5	0.15	2.0	No effect	No effect	Maternal abnormalities at necropsy	-
0.6	-	-	-	-	-	69% mortality; decreased maternal weight gain,[b] hematocrit[a], fetal weight[a]; fetal *stunting* increased[b]
1.0	0.3	4.0	No effect	No effect	86% mortality	-
1.5	0.45	6.0	-	No effect	100% mortality	-
2.0	0.6	8.0	10% mortality decreased maternal and fetal weights[a] live fetuses/ litter[b]	-	100% mortality	-
2.5	0.75	-	18% mortality decreased maternal and fetal weights[a] live fetuses/ litter[b]	-	-	-

[a]*Trend or tendency*, not significantly different from control values.
[b]Significantly different ($P \leq 0.05$) from control values (Duncan's multiple range test).

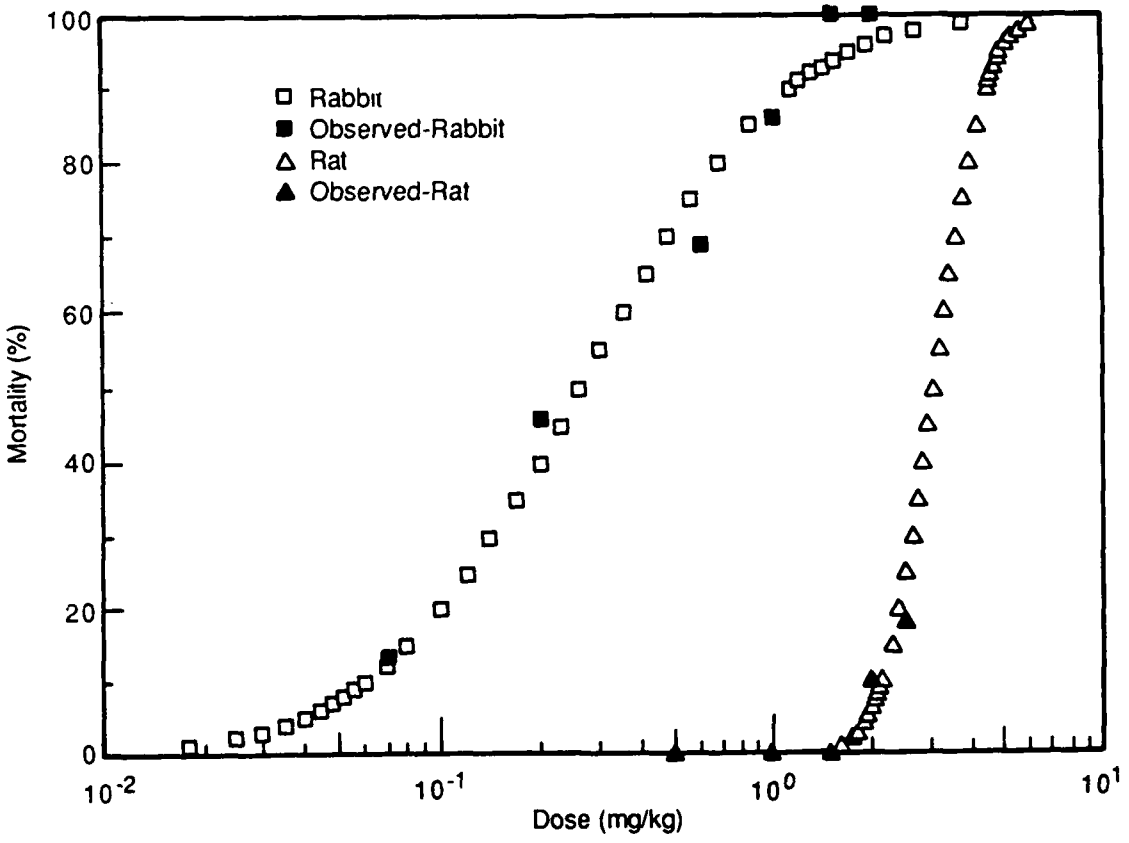

Figure 6. Results of Probit Analyses of Mortality Data from Dose-Range and Teratology Studies in Rats and Rabbits.

dose-range study, mortality rates followed a logical, progressive decrease from 100% at dose levels of 1.5 and 2.0 mg/kg to 14% at an administered dose of 0.07 mg/kg. In the 0.5 mg/kg group, the immediate cause of death of three rabbits was the delivery of the lewisite dose to the lungs; however, at necropsy, moderate to severe gastric lesions were evident in all of these animals. If death from dosing trauma had not intervened in the development of the lewisite syndrome, the mortality rate would have been 60%, which would provide a better point on the mortality curve between 69%, the rate observed at a dose level of 0.6 mg/kg and 46% at 0.2 mg/kg.

At necropsy, gastric lesions were the most common observations in both rats and rabbits that died from lewisite toxicity. Mucosal inflammation and hemorrhage were evident in both species, but were more pronounced in rabbits; edema, necrosis and mucosal sloughing were also observed in this species. At scheduled necropsy (5 and 11 days after cessation of dosing in rats and rabbits, respectively), the gastrointestinal tracts of the treated animals

appeared to be normal, on gross examination except, for one rat with a perforated ulcer and two rabbits with gastric inflammation and excessive amounts of peritoneal or pericardial fluid.

In rats, significant signs of maternal toxicity, such as depressed body weights and weight gains, were apparent only at dose levels that also caused death of the maternal animals. In the rabbit teratology study, body weight gains were decreased in the animals receiving the highest dose of lewisite (0.6 mg/kg) but were not depressed in rabbits of the 0.2 and 0.07 mg/kg dose groups, despite the fact that mortality was 46% and 14%, respectively. Another toxic symptom, the trend in decreased hematocrit values in these rabbits, may be attributed to gastric hemorrhage, changes in plasma volume and erythropoiesis during pregnancy (Eastman and Hellman, 1956) or to the periods of anorexia, particularly in rabbits of the highest dose group.

The litter, rather than the fetus was defined as the experimental unit for determining the significance of observations for fetal morphologic alterations (Kalter, 1974; Haseman and Hogan, 1975). Using this constraint, none of the variations involving reduced skeletal ossification in rat and rabbit fetuses were significant; the incidence of stunted rabbit fetuses in the highest dose group was statistically significant.

Results from the teratology study in rats demonstrated that daily doses of 1.5 mg of lewisite/kg, delivered to the pregnant animals for a period of 10 days, did not induce significant toxic or teratogenic effects in maternal rats or their fetuses. A comparison of these results with those of the dose-range study, indicates that 2.0 mg/kg might have been a better choice for the highest dose in the teratology study, although 11% maternal mortality, a trend in decreased maternal body weight, a significant reduction in the number of viable fetuses and a tendency toward fetal body weight reduction were observed at this higher dose level. The apparent differences resulting from exposure to 1.5 and 2.0 mg/kg suggest that toxic effects on fetal development would be observed only in litters of dams exhibiting significant signs of lewisite toxicity.

Results from the rabbit teratology study were statistically compromised by the small number of survivors which resulted from unanticipated mortality in all dose groups. It is evident that a "no effect level" (NOEL) for lewisite, delivered in 14 daily doses to maternal rabbits, lies below a dose level of 0.07 mg/kg. Observations of toxic effects in fetuses, which were also limited by the sample size, were not evident at the two lower dose levels of 0.07 and 0.2 mg/kg, but body weights of fetuses in the highest dose group (0.6 mg/kg) of the teratology study tended to be lower than control values and the incidence of stunted fetuses was significantly higher. Although statistical significance cannot be attached to all of these findings, these results suggest that signs of fetal toxicity were present at dose levels higher than those required to induce maternal mortality.

LITERATURE CITED

Adams, C. E. 1961. Artificial insemsination in rabbits. Commer. Rabbit Assoc. Tech. Bull. 1: 1-4.

Altman, P. L. and D. S. Dittmer. 1972. Biology Data Book, pp. 347, 350 and 351, FASEB, Bethesda, MD.

Aposhian, H. V. 1982. Biological chelation: 2,3-dimercapto-propanesulfonic acid and meso-dimercaptosuccinic acid. Adv. Enzyme Regulation 20: 301-319.

Barrow, M. V. and W. J. Taylor. 1969. A rapid method for detecting malformations in rat fetuses. J. Morphol. 127: 291-305.

Beaudoin, A. R. 1974. Teratogenicity of sodium arsenate in rats. Teratology 10: 153-158.

Cassarett, L. J. and J. Doull. 1986. Toxicology. The Basic Science of Poisons, 3rd Ed., Macmillan Publishers, New York, NY.

Dixon, W. J., M. D. Brown, L. Engleman, J. Frane, M. Hill, R. Jennrick, and J. Toporek (eds.). 1983. BMDP Biomedical Computer Programs. University of California Press, Berkeley, CA.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Dutkiewicz, T. 1977. Experimental studies on arsenic absorption routes in rats. Environ. Health Perspectives 19: 173-177.

Eastman, N. J. and L. H. Hellman. 1966. Williams Obstetrics, pp. 237-241. Appleton-Century-Crofts, New York, NY.

Ferm, V. H. and S. J. Carpenter. 1968. Malformations induced by sodium arsenate. J. Reprod. Fert. 17: 199-201.

Furia, T. E. (ed.). 1972. Handbook of Food Additives. CRC Press, Cleveland, OH.

Gates, M., J. W. Williams and J. A. Zapp. 1946. Arsenicals. Chemical Warfare Agents and Related Chemical Problems, Summary Technical Report of Division 9, National Defense Research Committee, Vol. 1, parts I and II, pp. 83-114. Washington, D.C.

Gregoire, A. T., R. W. Bratton, and R. H. Foote. 1958. Sperm output and fertility of rabbits ejaculated either once a week or once a day for 43 weeks. J. Anim. Sci. 17: 243-248.

Hafez, E. S. E. (ed.). 1970. Reproduction and Breeding Techniques for Laboratory Animals. Lea & Febiger, Philadelphia, PA.

Hagen, K. W. 1974. Colony husbandry, pp. 23-47. In: The Biology of the Laboratory Rabbit, S. T. Weisbroth, R. E. Flatt, and A. L. Kraus (eds.). Academic Press, New York, NY.

Haseman, J. K. and M. D. Hogan. 1975. Selection of the experimental unit in teratology studies. Teratology 12: 165-172.

Hood, R. D. and S. L. Bishop. 1972. Teratogenic effects of sodium arsenate in mice. Arch. Environ. Health 24: 62-65.

Hsu, C., H. V. Aposhian, M. Mershon, F. Brinkley and B. Hackley. 1982. Stability and anti-lewisite activity of dimercaptosuccinic acid and dimercapto-1-propanesulfonate. Fed. Proc. 41: 1569.

Kalter, H. 1974. Choice of the number of sampling units in teratology. Teratology 9: 257-258.

Kimmel, C. A., C. J. Price, B. M. Sadler, R. W. Tyl, and F. S. Gerling. 1985. Comparison of distilled water (DW) and corn oil (CO) vehicle controls from historical teratology study data. The Toxicologist 5: 185.

Kopf, R., D. Lorenz, and E. Salewski. 1964. Der Einfluss, von Thalidomid auf die Fertilitat von Ratten in Generations versuch uber zwei Generationen. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 247: 127-135.

Krause, H. and E. I. Grussendorf. 1979. Zur Therapie des Morbus Bowen mit 5-Fluor-Uracil-Salbe. Dermatol. beruf. umwelft. 27: 176-178.

Leonard, A. and R. R. Lauwerys. 1980. Carcinogenicity, teratogenicity and mutagenicity of arsenic. Mutation Res. 75: 49-62.

Lewis, R. L. and R. L. Tatken (eds.) 1980. Registry of toxic effects of chemical substances. National Institute of Safety and Health, Cincinnati, OH.

Lewis, R. L. and D. V. Sweet (eds.) 1983. Registry of toxic effects of chemical substances, P. 342. National Institute of Safety and Health, Cincinnati, OH.

Mohler, H. and J. Sorge. 1939. Chemical warfare materials. XII. Light absorption by nose and throat, lung and skin poisons in ultraviolet of short wave length. Helv. Chim. Acta 22: 235-239.

National Academy of Science. 1977. Medical and Biologic Effects of Environmental Pollutants, Arsenic. Washington, D.C.

Njielsen, H. C. and J. S. Torday. 1983. Anatomy of fetal rabbit gonads and sexing of fetal rabbits. Lab. Animal. 17: 148-150.

Palmer, A. K. 1977. Incidence of sporadic malformations, anomalies and variations in random bred laboratory animals, pp. 52-59. In: Methods in

Prenatal Toxicology, D. Neubert, H.-J. Merker, and T. E. Kwasigrock (eds.). Georg Thieme Publishers, Stuttgart.

Palmer, A. K. 1978. The design of subprimate animal studies, pp. 239-246. In: Handbook of Teratology, Volume 4, J. G. Wilson and F. C. Fraser (eds.). Plenum Press, New York, NY.

Perera, J. 1985. Lewisite: new gas weapon in Gulf War. New Scientist, 4 April, p. 8.

Perraud, J. 1976. Levels of spontaneous malformations in the CD rat and the CD-1 mouse. Lab. Anim. Sci. 26: 293-300.

Peters, R. A., L. A. Stocken and R. H. S. Thompson. 1945. British anti-lewisite (BAL). Nature 156: 616-619.

Rewick, R. T., M. L. Schumacher and D. L. Haynes. 1986. The uv absorption spectra of chemical agents and stimulants. Appl. Spectroscopy 40: 152-156.

Rosenblatt, D. H., T. A. Miller, J. C. Dacre, I. Muul and D. R. Cogley. 1975. Problem Definition Studies on Potential Environmental Pollutants, II. Physical, Chemical, Toxicological, and Biological Properties of 16 Substances. AD A030428. Technical Report 7509, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD.

SAS. 1985. SAS Users Guide: Statistics Version, Edition 5. SAS Institute, Inc., Gary, NC.

Shepman, P. M. and R. R. Schmidt. 1984. Corn oil modulates immune function: Altered postnatal immune function in mice following its prenatal administration. Teratology 29: 57A.

Siegel, S. 1965. Non-parametric Statistics for Behavioral Sciences. McGraw-Hill, New York, NY.

Springer, D.L. 1982. Perinatal effects of SRC, p. 15. In: Pacific Northwest Laboratory Annual Report for 1981 to the DOE Assistant Secretary for Environment, Part 1, Biomedical Sciences, PNL-4100. NTIS, Springfield, VA.

Staples, R. E. 1974. Detection of visceral alterations in mammalian fetuses. Teratology 9: A-37.

Staples, R. E. and V. L. Schnell. 1964. Refinements in rapid clearing technic in the KOH-Alizarin red-S method for fetal bone. Stain Technol. 39: 61-63.

Stertz, H. 1977. Routine examination of rat and rabbit fetuses for malformation of internal organs. Combination of Barrow's and Wilson Methods, pp. 113-125. In: Methods in Prenatal Toxicology, D. Neubert, H.-J. Merker, and T. E. Kwasigrock (eds.). Georg Thieme Publishers, Stuttgart.

Stuckhardt, J. L. and S. M. Poppe. 1984. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogen. Carcinogen. Mutagen. 4: 181-188.

Tesh, S. H. and J. M. Tesh. 1971. Artificial insemination in the rabbit and its use in teratogenic studies. Proc. Eur. Soc. Study Drug Tox. 12: 332-336.

van Julsingha, E. B. and C. G. Bennett. 1977. A dissecting procedure for the detection of anomalies in the rabbit foetal head, pp. 126-144. In: Methods in Prenatal Toxicology, D. Neubert, H.-J. Merker, and T. E. Kwasigrock (eds.). Georg Thieme Publishers, Stuttgart.

Wilson, J. G. and J. Warkany (eds.). 1965. Teratology Principles and Techniques. University of Chicago Press, Chicago, IL.

Windholz, M. (ed.). 1983. The Merck Index, 10th ed., Merck & Co., Inc., Rahway, NJ.

Zerbe, G.O. 1979. Randomization analysis of complete randomized design extended to growth and response curves. J. Am. Statistical Assoc. 79:215-221.

GLOSSARY

AI = Artificial insemination
AV = Artificial vagina

dg = Days of gestation

FDA = Federal Drug Administration

GC = Gas chromatography
GLP = Good Laboratory Practices

HP = Hewlett-Packard

IG = Intragastric
IP = Intraperitoneal
ISTD = Internal standard
IV = Intervenous

KRV = Kilham rat virus

LD_{50} = Median lethal dose

NFB = Neutral buffered formalin
NOEL = No observable effect level

PNL = Pacific Northwest Laboratory
PVM = Pneumonia virus of mice

RCV/SDA = Rat corona virus/sialodacryoadenitis virus
RH = Relative humidity

SC = Subcutaneous
SD = Standard deviation
SE = Standard error
SOP = Standard operating procedure

USAMRICD = U.S. Army Medical Research Institute of Chemical Defense

PERSONNEL LIST

Function	Name
Principal Investigators	P.L. Hackett L.B. Sasser
Facility Manager	M.T. Karagianes
Solution Preparation and Analysis	D.R. Kalkwarf C.W. Lindenmeier L.B. Sasser C. Veverka, Jr.
Animal Exposures and Evaluation	T.A. Breier J.A. Cushing J.J. Evanoff P.L. Hackett C.W. Lindenmeier M. Orgill R.L. Rommereim D.W. Shea M.L. Sours B.J. Willemsen R.C. Zangar
Animal Resource Center	E.L. Wierman
Health Evaluation	S.E. Rowe
Statistical Analyses	R.F. Buschbom

P.L. Hackett — 12/21/87
P.L. Hackett — Date

L.B. Sasser — 12-21-87
L.B. Sasser — Date

DISTRIBUTION

OFFSITE

Commander (33)
U.S. Army Biomedical Research
& Development Laboratory
Attn: SGRD-UBZ-C
Fort Detrick
Frederick, MD 21701-5010

Commander (2)
U.S. Army Medical Research &
Development Command
Attn: SGRD-PLE (RA V)
Fort Detrick
Frederick, MD 21701-5012

Commander (2)
U.S. Army Medical Research
Institute of Chemical
Defense
Attn: SGRD-UV-ZB
Aberdeen Proving Grounds, MD
21010-5425

U.S. Army Medical Research &
Development Command (1)
Attn: SGRD-RMI-S
Fort Detrick
Frederick, MD 21701-5012

Chemical Effects Information
Center (1)
Oak Ridge National Laboratory
P.O. Box X
Oak Ridge, TN 37831

Defense Technical Information
Center (DTIC) (1)
Attn: DTIC-DDAC
Cameron Station
Alexandria, VA 22304-6145

Technical Information
Center (2)
U.S. Department of Energy
P.O. Box 62
Oak Ridge, TN 37830-0062